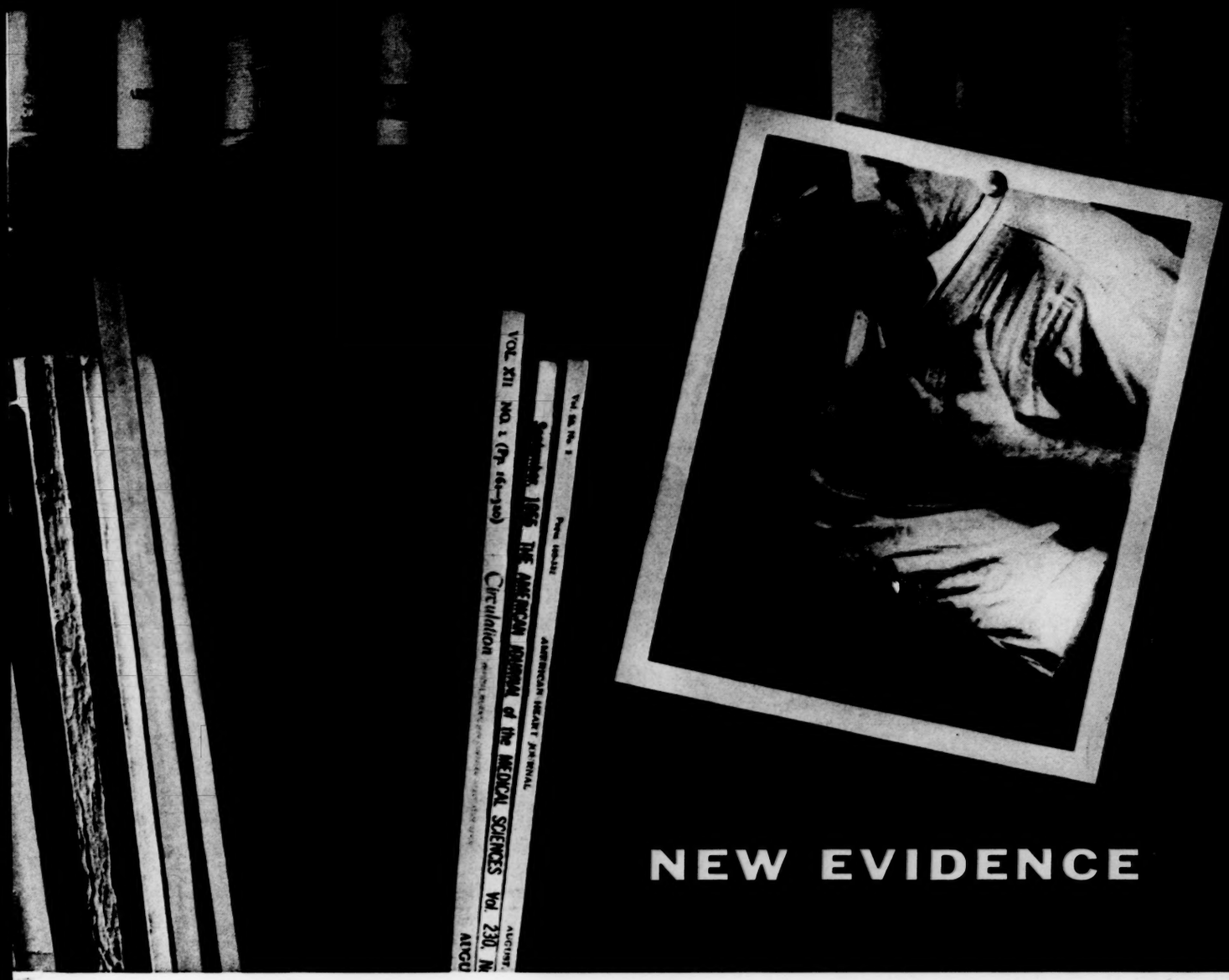
The American Journal of Medicine

The American Journal of Medicine

The American Journal of Medicine is published monthly by The American Journal of Medicine, Inc., 49 West 45th Street, New York 36, N. Y. Yearly Subscription, $12.00 *U. S. A.;* $13.00 *Canada;* $15.00 *Foreign, including Latin-American countries, Single Numbers* $2.00*; Symposia Numbers* $4.00. *Entered as Second Class Matter June* 28, 1946, *at the Post Office, New York, N. Y., and on June* 28, 1946, *at York, Pa., under the act of March* 3, 1879. *February,* 1956—*Volume* XX, *No.* 2.

MANUSCRIPTS: All manuscripts should be addressed to the Editorial Office of the Journal, 49 West 45th St., New York 36, N. Y. *Style for bibliography:* Doe, J. J. Treatment of hypertension. Am. J. Med., 6: 72, 1948.

Change of address must reach us one month preceding month of issue.



NEW EVIDENCE

How you can prevent attacks of angina pectoris

Three new studies have recently been added to the extensive investigation of Peritrate's effectiveness in preventing attacks of angina pectoris:

For some patients, state Rosenberg and Michelson, Peritrate "may mean the difference between complete, or almost complete, absence of symptoms, or a prolonged illness with much suffering." *Am. J. M. Sc. 230:254 (Sept.) 1955.*

"Impressive and sustained improvement" was also observed in a small number of patients treated by Kory *et al. Am. Heart J. 50:308 (Aug.) 1955.*

Among anginal prophylactic drugs evaluated by Russek's group "only this agent [Peritrate] appears worthy of the designation, 'long-acting coronary vasodilator.' " *Circulation 12:169 (Aug.) 1955.*

By prescribing Peritrate on a continuous daily dosage schedule (10 or 20 mg. 4 times a day) you can diminish the number and severity of attacks . . . reduce nitroglycerin dependence . . . increase exercise tolerance . . . improve abnormal EKG findings.

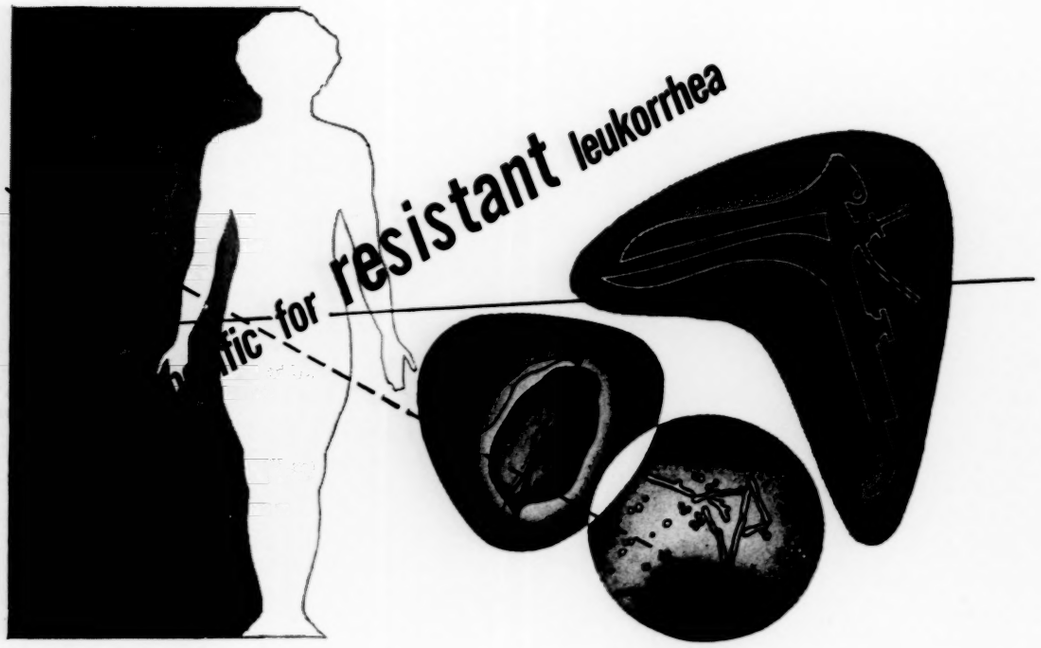
Usual dosage: 10 to 20 mg. *before meals* and at bedtime.

Five dosage forms for your convenience: *Peritrate* 10 mg. and 20 mg.; *Peritrate Delayed Action* (10 mg.) for extended protection at night; *Peritrate with Phenobarbital* (10 mg. with phenobarbital 15 mg.) where sedation is also required; *Peritrate with Aminophylline* (10 mg. with 100 mg. aminophylline) in cardiac and circulatory inadequacy.

Peritrate®

(BRAND OF PENTAERYTHRITOL TETRANITRATE)

WARNER-CHILCOTT



HIGHLY SUCCESSFUL — Extensive clinical investigations have yielded successful results with Milibis vaginal suppositories in 97 per cent of cases of trichomonal, monilial, bacterial and mixed vaginal infections.

RAPID RESPONSE —

In many instances, 10 Milibis vaginal suppositories, one inserted every other night, proves sufficient. In some cases, however, it was necessary to extend or repeat treatment or to increase the dose up to 2 suppositories daily for two weeks.

MILIBIS® VAGINAL SUPPOSITORIES

Supplied in boxes of 10, each suppository containing 0.25 Gm. of Milibis in a gelatin-glycerine base.

Winthrop LABORATORIES • NEW YORK 18, N. Y. • WINDSOR, ONT.

Milibis, trademark reg. U. S. Pat. Off., brand of glycobiarsol

CONTENTS

The American Journal of Medicine

No. XX FEBRUARY, 1956 No. 2

Editorial

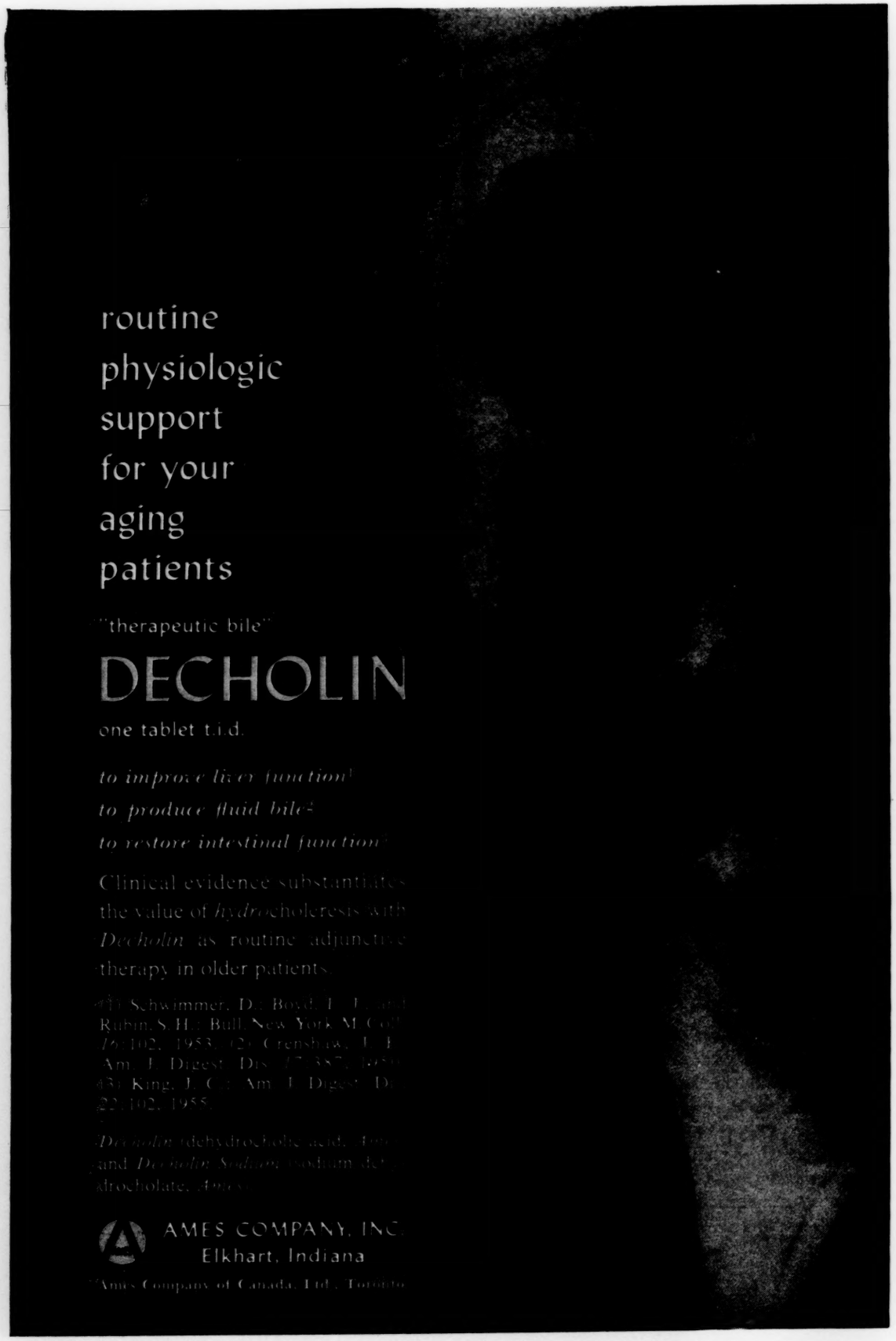
Clinical Studies

The authors have made the most of an opportunity to study under well controlled conditions the natural history of rheumatic fever as modified by prophylaxis and as not so modified. The present report deals with long term observations in 580 rheumatic subjects. It provides further evidence, if needed, of the incitant role of group A streptococcal infection as detected by titers of such antibodies as antistreptolysin O, antistreptokinase and antihyaluronidase. Of special interest are their observations on the etiologic relationships of streptococcal infection to post-treatment relapse in rheumatic fever and to "chronic" rheumatic fever. This latter state occurred in only 4.3 per cent of cases when fresh streptococcal infection was prevented, another significant argument for effective prophylaxis.

The etiologic relationship of Sydenham's chorea to infection with group A streptococci and to rheumatic fever has long been a matter of controversy, not altogether resolved by this study. Despite a puzzling chronology, however, this analysis of streptococcal antibody titers supports the generally accepted relationship, even in "pure" chorea.

It is generally appreciated that antibiotic-resistant strains of staphylococci are becoming more prevalent but the data presented by Dr. Spink and his associates are startling and disturbing to say the least. They leave no doubt that the personnel and inpatients of hospitals, particularly of hospitals using large quantities of antibiotics (and which do not), have a high and increasing incidence of nasopharyngeal and skin infection with antibiotic-resistant strains of staphylococci. That such carriers present a hazard, particularly in respect to wound infection in surgical cases, needs no emphasis, and the desirability of more effective prevention of cross infection also is evident. This report, in any case, deserves attention.

Contents continued on page 5


routine
physiologic
support
for your
aging
patients
"therapeutic bile"
DECHOLIN
one tablet t.i.d.
to improve liver function[1]
to produce fluid bile[2]
to restore intestinal function[3]
Clinical evidence substantiates
the value of hydrocholeresis with
Decholin as routine adjunctive
therapy in older patients.
Rubin, S.H.: Bull. New York M. Co
Jo. 102, 1953. (2) Crenshaw, L. E
Am. J. Digest. Dis. 17: 387, 1950
(3) King, J. C.: Am. J. Digest. Di
22: 102, 1955.
Decholin (dehydrocholic acid), Am
and Decholin Sodium (sodium de
drocholate), Ames
AMES COMPANY, INC.
Elkhart, Indiana
Ames Company of Canada, Ltd., Toronto

CONTENTS continued–February 1956

VOLUME TWENTY NUMBER TWO

Contents continued on page 7

AN ADVANCE IN ACTH THERAPY

One injection is effective for 24 to 72 hours or more

CORTROPHIN*-ZINC†

By minimizing the therapeutic "ups and downs" which may occur during therapy with ACTH-in-gel, truly long-acting Cortrophin-Zinc provides a smooth corticotropin action for 1 to 3 days.

Cortrophin-Zinc is convenient to administer. It is an aqueous suspension which flows easily through a 24-gauge needle, eliminating preheating, clogging syringes, and heavy-gauge needles to add to the pain.

Supplied: In 5 cc vials, each cc containing 40 U.S.P. units of corticotropin with 2 mg of zinc.

A development of
Organon INC.
ORANGE, N. J.

*T.M.—Cortrophin †Organon brand of Corticotropin-Zinc Hydroxide—Patent Pending. Available in other countries as Cortrophine-Z.

CONTENTS continued–February 1956

VOLUME TWENTY NUMBER TWO

Contents continued on page 9

CONTENTS continued–February 1956

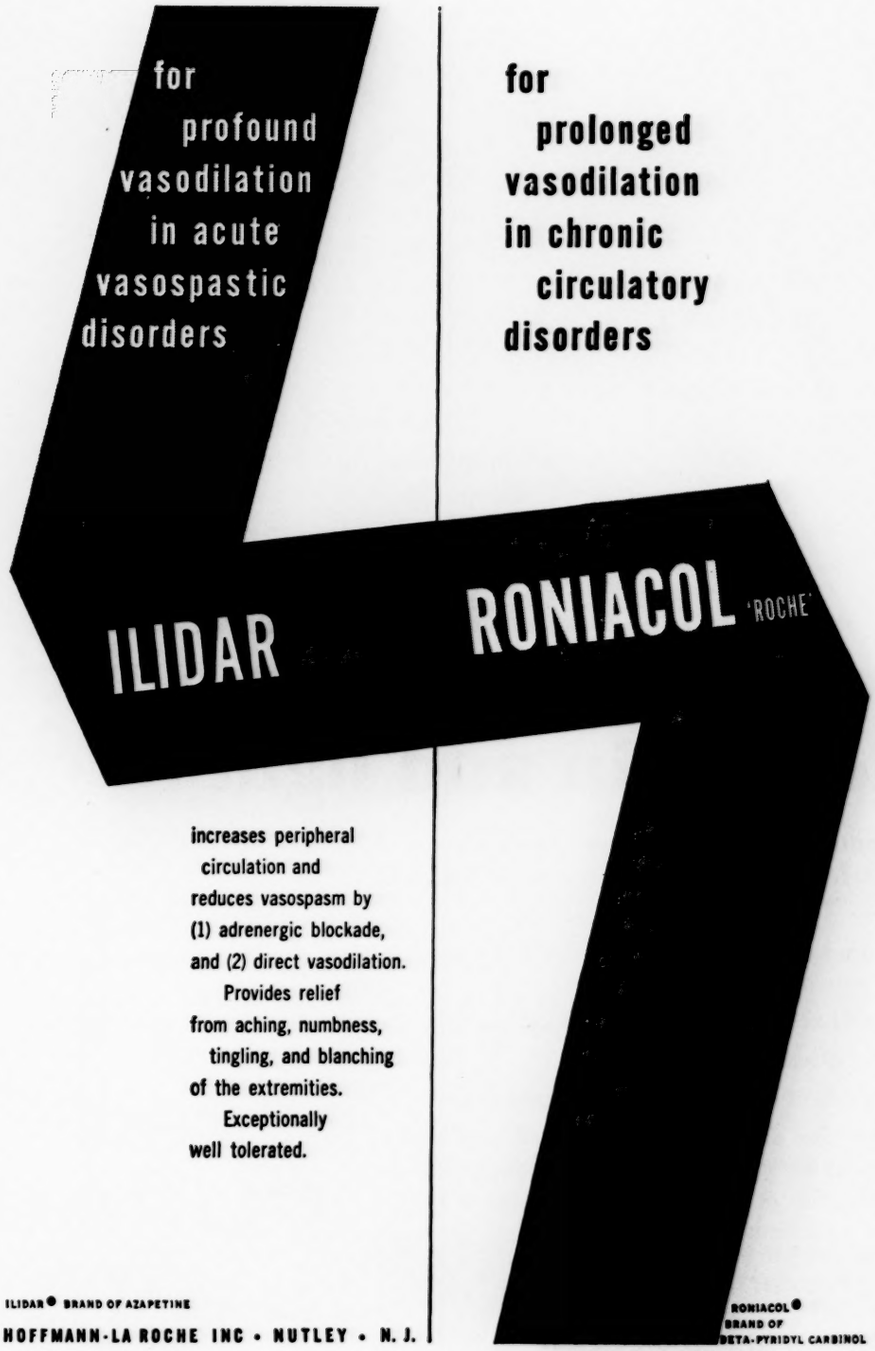
VOLUME TWENTY NUMBER TWO

Clinic on Psychosomatic Problems

Case Reports

Advertising Index on 3rd Cover

Change of address must reach us one month preceding month of issue.


for
profound
vasodilation
in acute
vasospastic
disorders
for
prolonged
vasodilation
in chronic
circulatory
disorders
ILIDAR
RONIACOL
'ROCHE'
increases peripheral
circulation and
reduces vasospasm by
(1) adrenergic blockade,
and (2) direct vasodilation.
Provides relief
from aching, numbness,
tingling, and blanching
of the extremities.
Exceptionally
well tolerated.
ILIDAR® BRAND OF AZAPETINE
HOFFMANN-LA ROCHE INC • NUTLEY • N. J.
RONIACOL®
BRAND OF
BETA-PYRIDYL CARBINOL

New urethral suppositories relieve pain and fight infection

For the patient, "The treatment is shorter and more comfortable than with the conventional method..."*

- suppository melts and releases anesthetic agent to relieve pain and burning *rapidly*
- kills most bacteria common to urinary tract
- easy for patient to insert at home
- *safe*—irritation rare in over 340 reported cases*
- *proved*—"The suppository method of medication has proved its worth."*. . . in bacterial (granular) urethritis; for prophylaxis and pain-relief before and after instrumentation.

to prevent cross-infection...

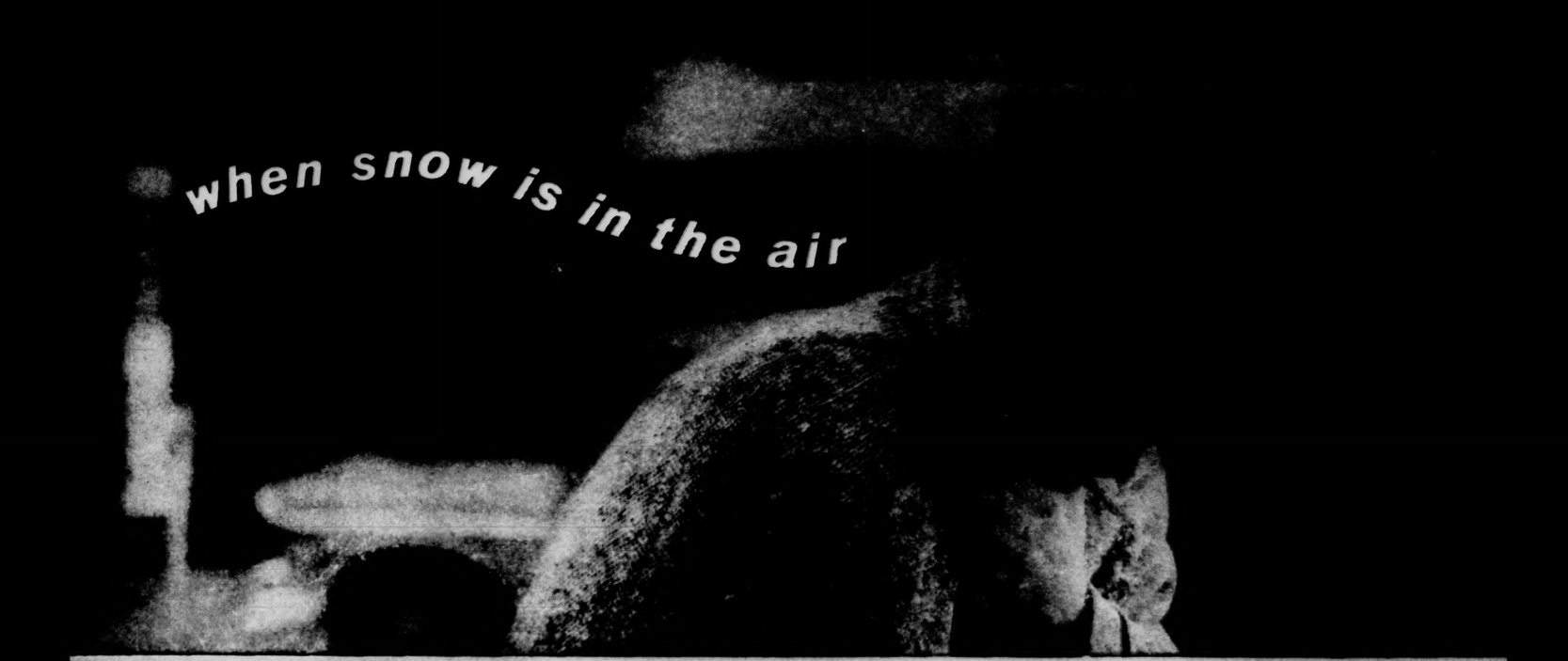
FURACIN® vaginal Suppositories are used with Furacin Urethral Suppositories to prevent cross-infection from the vagina. Hermetically sealed in yellow foil. Box of 12.

*Youngblood, V. H.: J. Urol. *70*:926, 1953.

FURACIN®
BRAND OF NITROFURAZONE
urethral
SUPPOSITORIES

Each Furacin Urethral Suppository contains 0.2% Furacin (brand of nitrofurazone) and 2% diperodon HCl in a water-miscible base. Hermetically sealed in silver foil. Box of 12.

EATON LABORATORIES, Norwich, N.Y. **NITROFURANS** a new class of antimicrobials neither antibiotics nor sulfas



time for

Tyzine®

brand of tetrahydrozoline hydrochloride

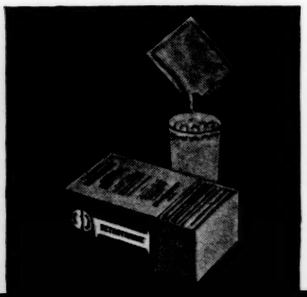
nasal decongestion in minutes for hours

"a new and superior topical vasoconstrictor,"* with effectiveness lasting even up to 6 hours, without rebound engorgement. Odorless, tasteless, free of sting, burn, irritation, and CNS stimulation.

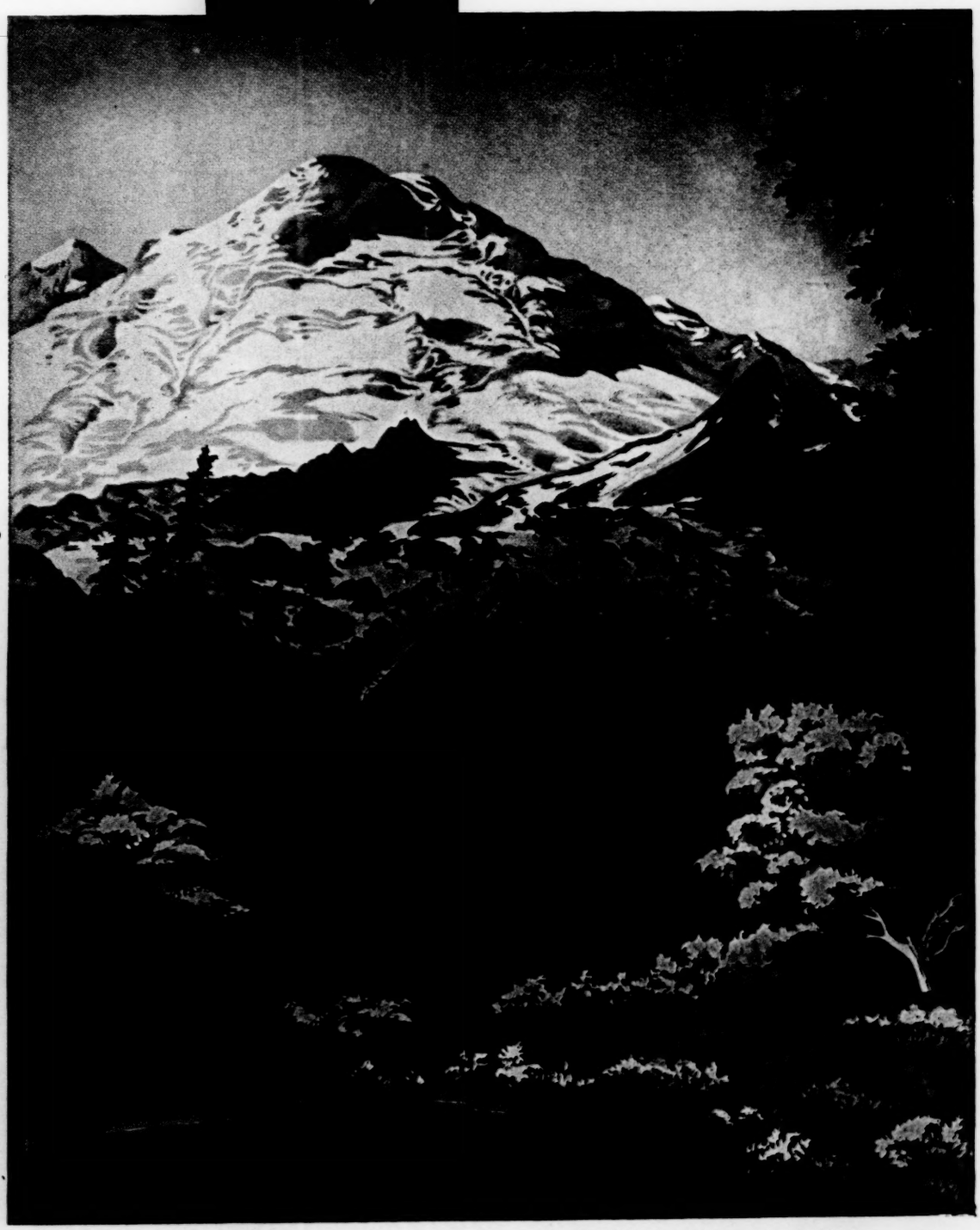
Supplied: TYZINE Nasal Spray, in plastic bottles, 15 cc. TYZINE, 0.1%. TYZINE Nasal Solution, in 1-oz. dropper bottles, 0.1%. TYZINE Pediatric Nasal Drops, in 1/2-oz. bottles, 0.05%, with calibrated dropper for precise dosage.

Menger, H. C.: New York J. Med. 55:812, 1955

PFIZER LABORATORIES *Division, Chas. Pfizer & Co., Inc. Brooklyn 6, New York* Pfizer



a holiday



from fatigue

Betasyamine® . . . for a holiday from fatigue . . . in your "well" patients who feel sick.

Your tired patients: Betasyamine has been included in the recovery and rehabilitation programs of many common conditions characterized by chronic fatigue, impaired neuromuscular function, and anxiety tension states. It has been clinically established[1, 2, 3, 4] to be of distinct benefit in relieving exhaustion, re-establishing muscle tone, creating a new mood of optimism. Low energy states have been linked with subnormal muscle and nerve phosphocreatine readings.[1] Betasyamine, containing betaine and glycocyamine, precursors of phosphocreatine, steps up these values to normal, thus tending to restore and maintain the dynamic energy balance.

Containing no unphysiologic sedative or stimulant drug, Betasyamine is true replacement therapy; it offers promise of—a holiday from fatigue—wherever increased burdens and stresses have undermined the energy reserve.

Average Dosage: 1 Effervescent Packet; 1 tablespoonful Emulsion; or 5 Tablets three times daily at mealtimes.

Supplied: Effervescent Packets (new) — 24's; Emulsion 16 fl. oz.; Tablets — 200's.

References: 1. Dixon, H. H., and others: West. J. Surg. *62*:338 (June) 1954. • 2. Jones, C. H.: (in press). • 3. Watkins, A. L.: New England J. Med. *248*:621 (April 9) 1953. • 4. Aldes, J. H.: Bull. Biol. Sciences Foundation *1*:4 (April) 1954.

Amino Products Division
International Minerals & Chemical Corp. • Chicago • San Francisco • Los Angeles

NOW IN TWO POTENCIES

for more precise dosage

NEW 1 mg. tablet | 5 mg. tablet

MOST POTENT ANTI-RHEUMATIC

Sterane*

Both tablets are deep-scored and of the [illegible] for ease of handling and breaking by arthritic fingers.

anti-rheumatic/anti-allergic/anti-inflammatory

supplied: 1 mg. oral tablets, bottles of 100.
White, 5 mg. oral tablets, bottles of 20 and 100.

PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
Brooklyn 6, New York

brand of [illegible]

A sensible approach to insomnia—

By relieving nervous tension, Noludar 'Roche' usually permits the patient to fall asleep naturally. Noludar is a gentle sedative-hypnotic; it is not a barbiturate and not habit-forming. 50-mg tablets for sedation; 200-mg tablets for insomnia.

Noludar® -- brand of methyprylon

these are

the reasons for

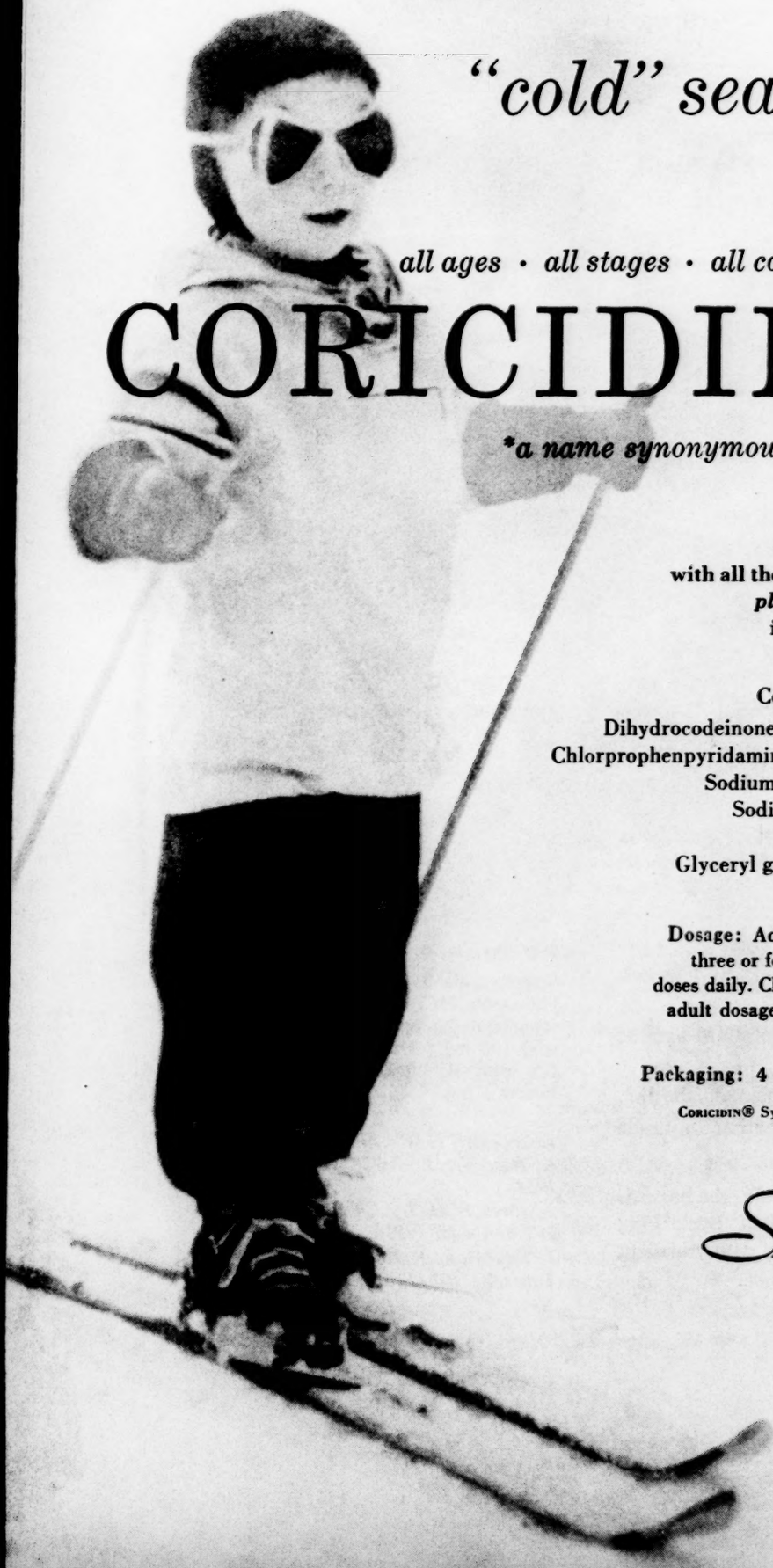
Gantricillin

It provides Gantrisin® PLUS penicillin... for well-tolerated, wide-spectrum anti-bacterial therapy...in tablets of two strengths -- Gantricillin®-300 'Roche' for severe cases; Gantricillin (100) for mild cases -- and in an easy-to-take suspension for children -- Gantricillin (acetyl)-200.

Hoffmann - La Roche Inc • Nutley • N.J.

it's the

"cold" season...



all ages · all stages · all coughs · respond to

CORICIDIN* *Syrup*

a name synonymous with cold control

one versatile preparation
with all the advantages of CORICIDIN
***plus* superior cough control**
in a pleasant-tasting syrup

Each teaspoonful (5 cc.) of
CORICIDIN Syrup* contains:

Dihydrocodeinone bitartrate	1.67 mg.
Chlorprophenpyridamine maleate	2 mg.
Sodium salicylate	225 mg.
Sodium citrate	120 mg.
Caffeine	30 mg.
Glyceryl guaiacolate	30 mg.

*Exempt narcotic.

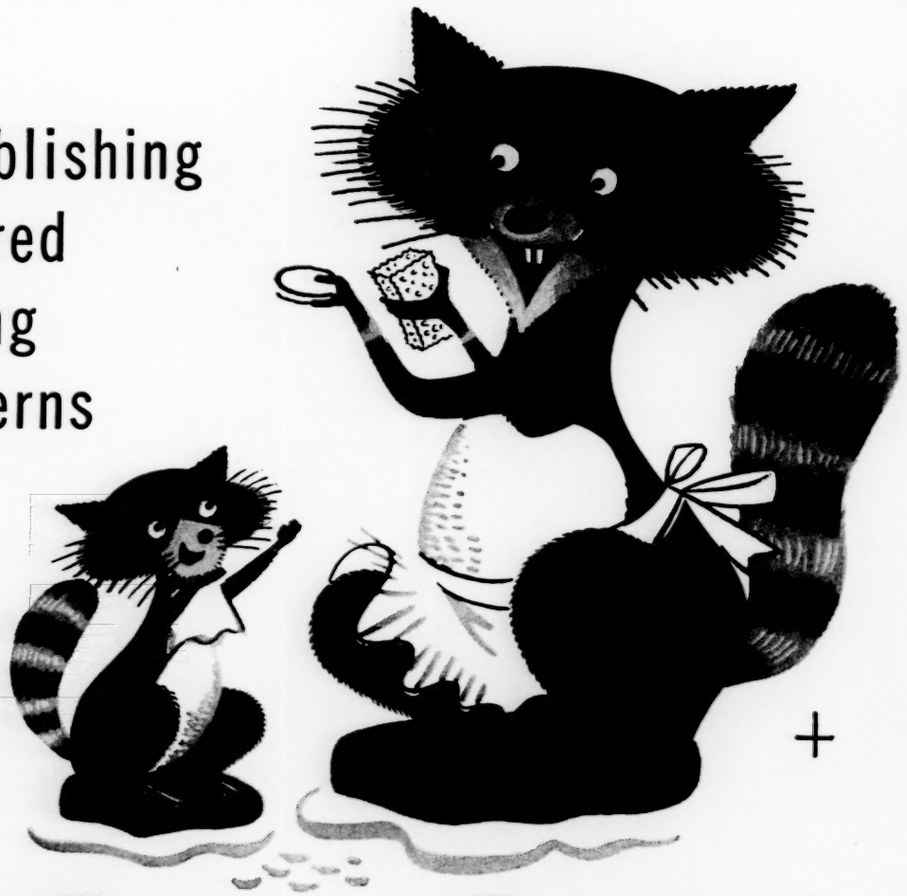
Dosage: Adults—One teaspoonful every three or four hours not exceeding four doses daily. Children 6-12 years—One-half adult dosage. Younger children—Adjust dosage according to age.

Packaging: 4 oz., pint, and gallon bottles.

CORICIDIN® Syrup, brand of antitussive-analgesic.

Schering

CN-J-54



establishing
desired
eating
patterns

Obedrin®

and the 60-10-70 Basic Plan

In the development of good eating habits, medication is important, not only in initiating control, but also in maintaining normal weight.[1,2,3]

Obedrin contains:

- Methamphetamine for its anorexigenic and mood-lifting effects.
- Pentobarbital as a balancing agent, to guard against excitation.
- Vitamins B_1 and B_2 plus niacin to supplement the diet.
- Ascorbic acid to aid in the mobilization of tissue fluids.

Since Obedrin contains no artificial bulk, the hazards of impaction are avoided. The 60-10-70 Basic Plan provides for a balanced food intake, with sufficient protein and roughage.

Formula

Semoxydrine HCl (Methamphetamine HCl) 5 mg.; Pentobarbital 20 mg.; Ascorbic acid 100 mg.; Thiamine HCl 0.5 mg.; Riboflavin 1 mg.; Niacin 5 mg.

1. Eisfelder, H.W.: Am. Pract. & Dig. Treat., 5:778 (Oct., 1954).
2. Sebrell, W.H., Jr.: J.A.M.A., 152:42 (May, 1953).
3. Sherman, R.J.: Medical Times, 82:107 (Feb., 1954).

Write for 60-10-70 Menu pads, weight charts, and samples of Obedrin.

THE S. E. MASSENGILL COMPANY
BRISTOL, TENNESSEE

NEW! 'Tetrazets'

BACITRACIN-TYROTHRICIN-NEOMYCIN-BENZOCAINE TROCHES

broader attack to overcome minor throat irritations

'TETRAZETS' quickly relieve minor mouth and throat irritations

It's new—a single troche containing 3 potent antibiotics (bacitracin, tyrothricin, neomycin) to combat afebrile oral infections.

'TETRAZETS' offer you the ideal topical treatment of minor irritations of the oral cavity.

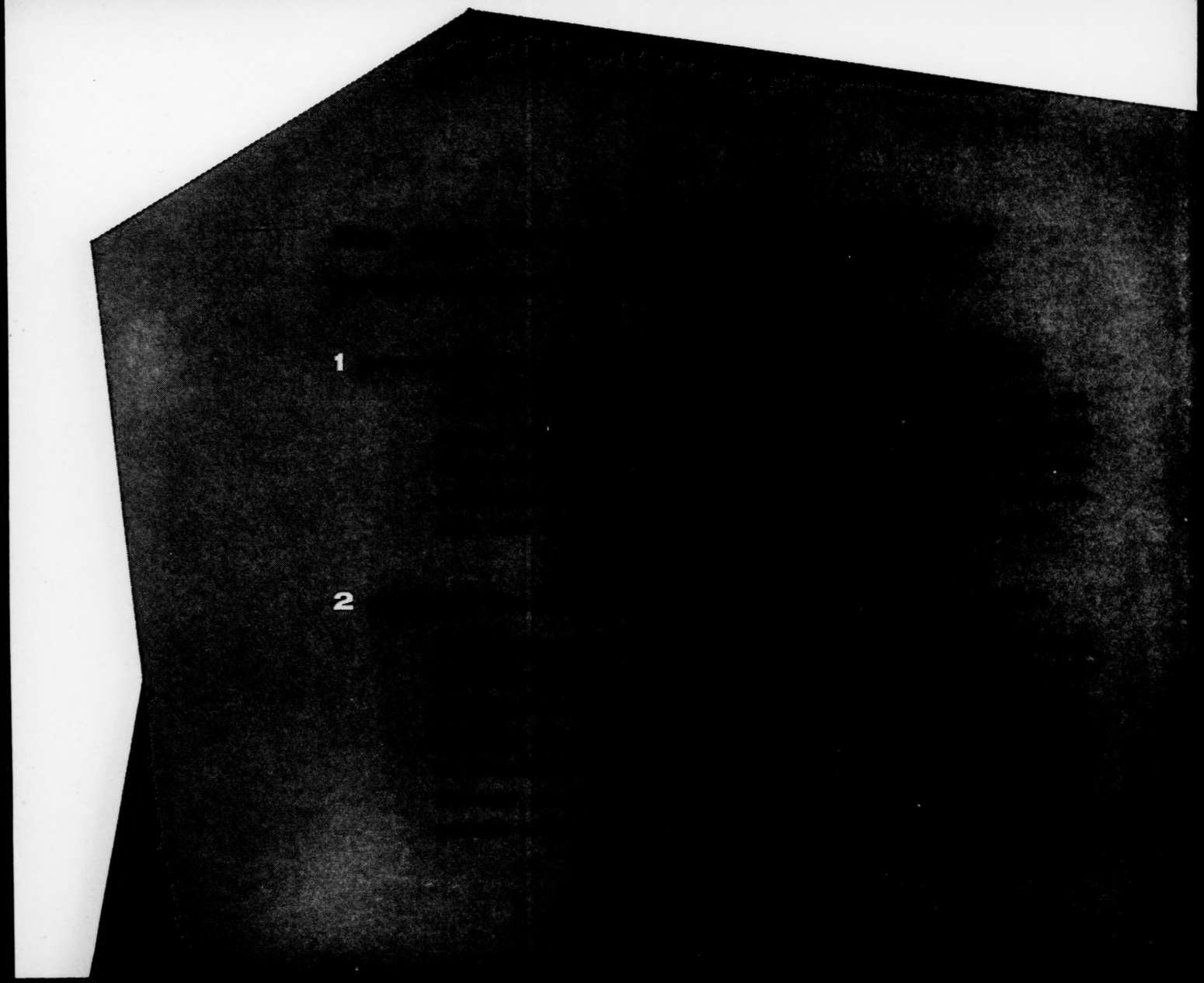
In deep-seated infections, such as Vincent's infection, tonsillitis and streptococcus sore throat, 'TETRAZETS' may be used as an adjuvant to parenteral antibiotics.

Before and after tonsillectomies, 'TETRAZETS' help combat secondary invaders.

Supplied: In vials of 12. Each 'TETRAZETS' troche contains 50 units of zinc bacitracin, 1 *mg.* tyrothricin, 5 *mg.* neomycin sulfate with 5 *mg.* benzocaine.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.



Mysteclin

STECLIN-MYCOSTATIN

(SQUIBB TETRACYCLINE-NYSTATIN)

A PARTIAL LIST OF INDICATIONS FOR MYSTECLIN

It is impossible to predict with certainty in which patients clinical moniliasis may develop as a result of broad spectrum antibiotic therapy. However, the added protection afforded by Mysteclin against monilial superinfection is especially important in patients who are debilitated, elderly, or diabetic, or when antibiotic therapy must be prescribed in high dosage or for prolonged periods. It is also important in infants (particularly prematures), as well as in patients for whom concomitant cortisone or related steroid therapy is prescribed, or in subjects who have developed a monilial complication on previous broad spectrum therapy.

Mysteclin is particularly useful in women, inasmuch as they not infrequently develop vulvovaginal moniliasis after treatment with ordinary broad spectrum antibiotics; this is especially common in women who are pregnant or diabetic.

SQUIBB

NOW

Ferrous Iron with Vitamin C

for simple
specific
rapid
economical

correction of iron deficiency anemias

"Optimal absorption of iron is best assured by administering it in the ferrous form with ascorbic acid . . ."*

"CYTOFERIN"—*the logical combination for iron therapy.*

- Iron in the readily absorbed ferrous form.
- Vitamin C to maintain acidity in the upper intestinal tract for greater iron absorption.
- Vitamin C as a reducing agent for the ferric iron obtained from food.
- Vitamin C for hypochromic microcytic anemias, which will not respond to oral iron therapy alone.

"CYTOFERIN"—*the direct approach to greater iron absorption.*

Each tablet contains:
Ferrous sulfate exsic. (3 gr.) . . . . . 200 mg.
Vitamin C (ascorbic acid) . . . . . 150 mg.

Dosage: 1 tablet two or three times daily, taken preferably with meals.

Supplied: No. 705, bottles of 100 and 1,000.

NEW PRODUCT

*Sacks, M. S.: Ann. Int. Med. *42*:458 (Feb.) 1955.

Ayerst

Ayerst Laboratories • New York, N. Y. • Montreal, Canada

5613

PHOTO CREDIT: COURTESY CHICAGO HISTORICAL SOCIETY, J. C. KEOGH

if
your
patients
remember
this...
they
may need

new NEOBON's* 4 factors, plus 1

(for those over 41)

* Gonadal Hormone Replacement
  (balanced combination of ethinyl estradiol and methyl testosterone)
* Hematinic Component
  (iron plus 7 other hematopoietic factors)
* Digestant Enzyme Replacement
  (helps insure adequate digestion)
* Nutritional Supplement
  (9 important minerals, 11 essential vitamins)

and the exclusive "plus 1" factor

* Protein Improvement
  (with lysine, essential amino acid commonly lacking in geriatric diets)

for 5 common problems of aging

Literature? Write... ℞ OERIG Chicago 11, Illinois

Erythromycin in treatment of abscess

6/21/55

DISCHARGE SUMMARY

On 5/23/55 this patient (colored female, age 24) underwent an excisional biopsy of a breast tumor. On 5/24 tumor was removed and patient discharged from hospital on following day.

On 6/3/55 patient was readmitted because of purulent discharge from wound. On 6/3 a hemolytic Staph. aureus (coag. +) was isolated from abscess with the following disk sensitivities: penicillin, 1.5 units; erythromycin, 10 mcg; tetracycline, 10 mcg. Patient was placed on penicillin, 600,000 units b.i.d. for 10 days. On this schedule patient improved but progress was unsatisfactory and wound continued to discharge small amount of purulent material.

On 6/13 penicillin was discontinued and erythromycin started in dosage of 200 mgm. q.i.d. By 6/17 the discharge had stopped and wound was completely healed by 6/19. Erythromycin was continued until the patient was discharged from hospital on 6/21. Temp. was normal throughout hospital stay.

Final diagnosis: breast abscess due to Staph. aureus.

Result: rapid and complete recovery on erythromycin following failure of penicillin.

Communication to Abbott Laboratories.

specific against coccic infections

Now, you can prescribe an antibiotic (*Filmtab* ERYTHROCIN) that provides *specific therapy* against staph-, strep- or pneumococci. Since these organisms cause most bacterial respiratory infections (and since they are the very organisms most sensitive to ERYTHROCIN) doesn't it make good sense to prescribe ERYTHROCIN when the infection is coccic?

filmtab®

Erythrocin®
(Erythromycin, Abbott)
STEARATE

with little risk of serious side effects

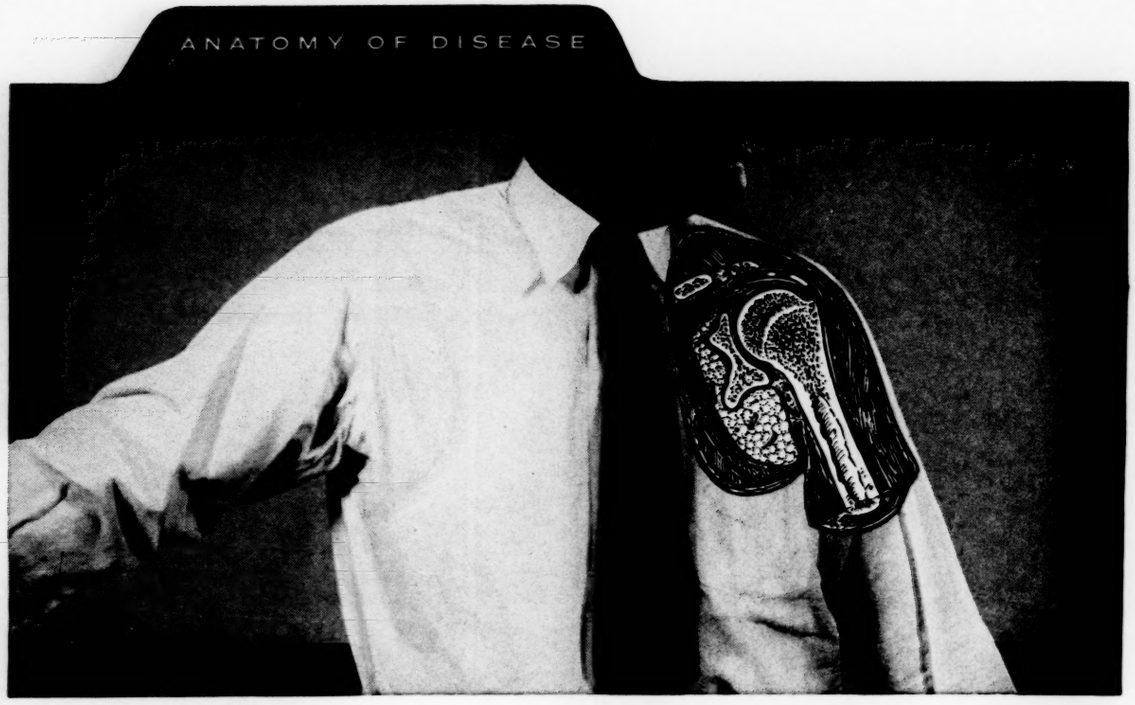
Since ERYTHROCIN is inactive against gram-negative organisms, it is less likely to alter intestinal flora—with an accompanying low incidence of side effects. Also, your patients seldom get the allergic reactions sometimes seen with penicillin. Or loss of accessory vitamins during ERYTHROCIN therapy. *Filmtab* ERYTHROCIN (100 and 250 mg.), bottles of 25 and 100. Abbott

filmtab®

Erythrocin®
(Erythromycin, Abbott)
STEARATE

® Filmtab—Film sealed tablets; patent applied for

601105



now . . . *reinforced anti-inflammatory action*

for better results in rheumatic and arthritic conditions

Armyl+F

Armyl + F greatly reinforces the antiarthritic and antirheumatic action of the salicylates. Synergistic action of the combination of agents in Armyl + F produces significantly better patient response with an extremely low dose of corticoid.

Each Armyl + F capsulette contains:

Compound F (hydrocortisone-free alcohol) ..... 2.0 mg.
Potassium Salicylate (5 gr.) .... 0.30 Gm.
Potassium Para-aminobenzoate (5 gr.) ..... 0.30 Gm.
Ascorbic Acid ..... 50 mg.

Bottles of 50 capsulettes.

but when the salicylates alone are enough

Armyl® for high salicylate blood levels . . . relief of pain . . . antihemorrhagic protection.

Each enteric-coated tablet contains:

Sodium Salicylate (5 gr.) ..... 0.3 Gm.
Sodium Para-aminobenzoate (5 gr.) ..... 0.3 Gm.
Ascorbic Acid (50 mg.) .....0.05 Gm.

Bottles of 100. Also available: Armyl with ½ gr. Phenobarbital; Armyl Sodium-Free; Armyl Sodium-Free with ½ gr. Phenobarbital.

THE ARMOUR LABORATORIES
A DIVISION OF ARMOUR AND COMPANY • KANKAKEE, ILLINOIS

"more than mere symptomatic relief..."[1]

Biomydrin® NASAL SPRAY

*"penetration makes the difference"

. . . in nasal infection and allergy Biomydrin penetrates crypts and sinuses because it is **mucolytic**

Biomydrin combats infection because it is **bactericidal**

Biomydrin relieves congestion because it is **vasoconstrictive** and **anti-allergic**

The Biomydrin Formula

Thonzonium bromide	0.05%	*mucolytic penetrating*
Neomycin sulfate Gramicidin	0.1% 0.005%	*bactericidal*
Thonzylamine HCl	1.0%	*antiallergic*
Phenylephrine HCl	0.25%	*decongestive*

½ oz. plastic atomizer or dropper bottle
Biomydrin has a yellow cap

Whenever added anti-inflammatory action is desired

Biomydrin·F

(WITH HYDROCORTISONE ALCOHOL, 0.02%.)

½ oz. plastic atomizer
Biomydrin F has a red cap

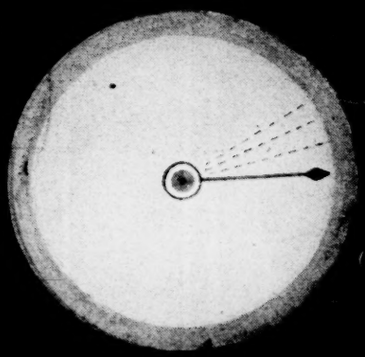
1. Lazar, A. M., and Goldin, M.: Eye, Ear, Nose & Throat Monthly *32*:512, 1953.

NEPERA CHEMICAL CO., INC.
Pharmaceutical Manufacturers
Nepera Park, Yonkers 2, N. Y.

B-1178-M

Steraject®

portable



available in seconds

compactly sterile

for home, office & hospital use

The new Steraject with one-piece cartridge-sterile needle assembly

Pfizer

because
your allergic patients
need a lift
a new R_x . . .

Plimasin®

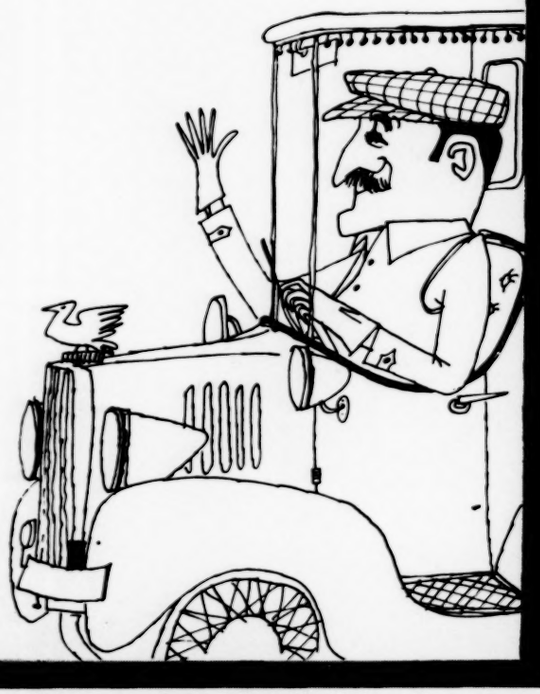
(tripelennamine hydrochloride and methyl-phenidylacetate CIBA)

new, mild stimulant
and antihistamine

boost their spirits . . . relieve their allergic symptoms

So often the allergic patient is tired, irritable, depressed—mentally and physically debilitated. Frequently, antihistaminic agents themselves are sedative, adding to this already fatigued and disconsolate state.

Plimasin, because it combines a proved antihistamine with a new, mild psychomotor stimulant, overcomes depression and fatigue while it achieves *potent* antiallergic effects. Its new stimulant component—Ritalin—is totally different from amphetamine: smoother, gentler in action, devoid of pressor effect.



DOSAGE: *One or 2 tablets as required.*

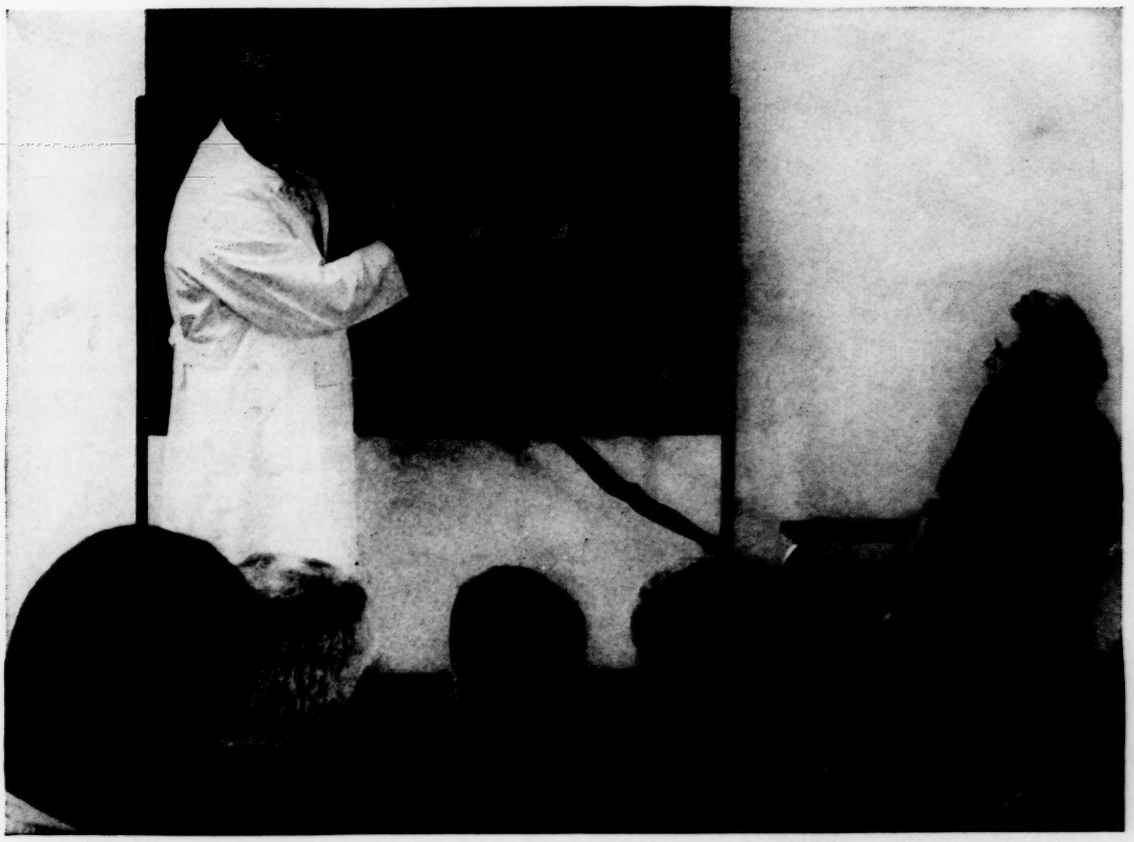
Each Plimasin tablet contains 25 mg. Pyribenzamine® hydrochloride (tripelennamine hydrochloride CIBA) and 5.0 mg. Ritalin® (methyl-phenidylacetate CIBA).

CIBA SUMMIT, N. J.

2/2191M

MEDICAL HORIZONS TV **Monday P.M.** *Sponsored by* CIBA **ABC-TV**

adjusts anticoagulant-depressed prothrombin time



MAJOR ADVANTAGES: Action detectable within 15 minutes, prothrombin time normalized within 4 to 12 hours, bleeding checked in 3 to 6 hours.

EMULSION OF Mephyton®

(VITAMIN K_1 INJECTION, MERCK)

"It is shown that oil-soluble vitamin K_1 is more effective than any other agent now available in combating drug-induced hypoprothrombinemia."[1] However, the usefulness of Mephyton lies not only in overcoming bleeding emergencies, but also in adjusting upwards to safe therapeutic levels unduly prolonged prothrombin times. Mephyton permits the clinician to do this easily and without gross changes in the regular anticoagulant dosage. . . ."no untoward effects have been observed to follow the administration of vitamin K_1."[1]

INDICATIONS: Hypoprothrombinemia due to Dicumarol®, Cumopyran®, Tromexan®, Hedulin®, 'Dipaxin', warfarin and other phenylindanediones; also when due to antibiotics, salicylates, obstructive jaundice, hepatic disease, and impaired gastrointestinal absorption.

SUPPLIED: In boxes of six 1-cc. ampuls, 50 mg. of vitamin K_1 per cc.

1. Gamble, J. R., *et al.*, *Arch. Int. Med.* 95:52, January 1955.

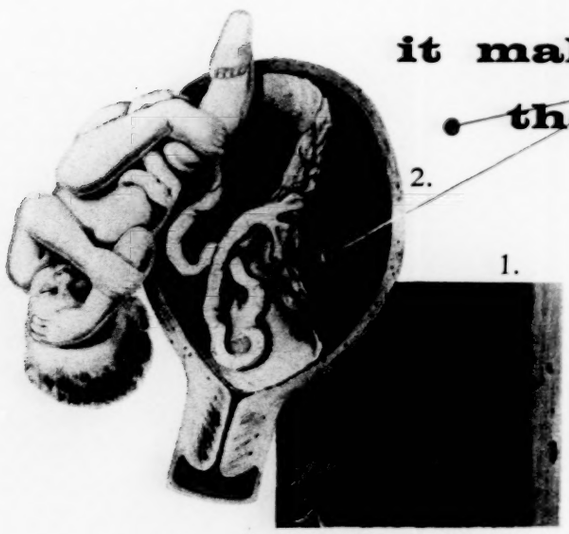
SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.

95% FETAL SALVAGE

with HESPER-C

it makes the difference



1. Increased fragility of the uterine capillaries leads to an effusion of blood into the decidua basalis. This is the beginning of
2. Abruptio placentae

From the ripe golden beauty of the fruit comes an influence for the finest fruition of all—the ripe offspring of love—children of today, men of tomorrow. A synergistic combination of hesperidin and ascorbic acid, Hesper-C is recommended as an integral part of any regimen for fetal salvage.[1] Maintaining capillary integrity during the critical months[2] guards against abruptio placentae. In 100 patients whose 420 previous pregnancies resulted in 95% fetal wastage, the addition of Hesper-C to current therapy completely reversed the figure and resulted in 95% fetal salvage.[3]

Remember Rx Hesper-C along with your usual therapy—it makes the difference. Maintain the integrity of the capillaries throughout pregnancy.

DOSAGE: Initially 6 capsules or more per day for the first week. Then 4 capsules daily.

SUPPLIED: Hesper-C (hesperidin 100 mg. and ascorbic acid 100 mg.) capsules are available in bottles of 100 and 1000.

ON YOUR PRESCRIPTION ONLY

Send for samples and reprints.

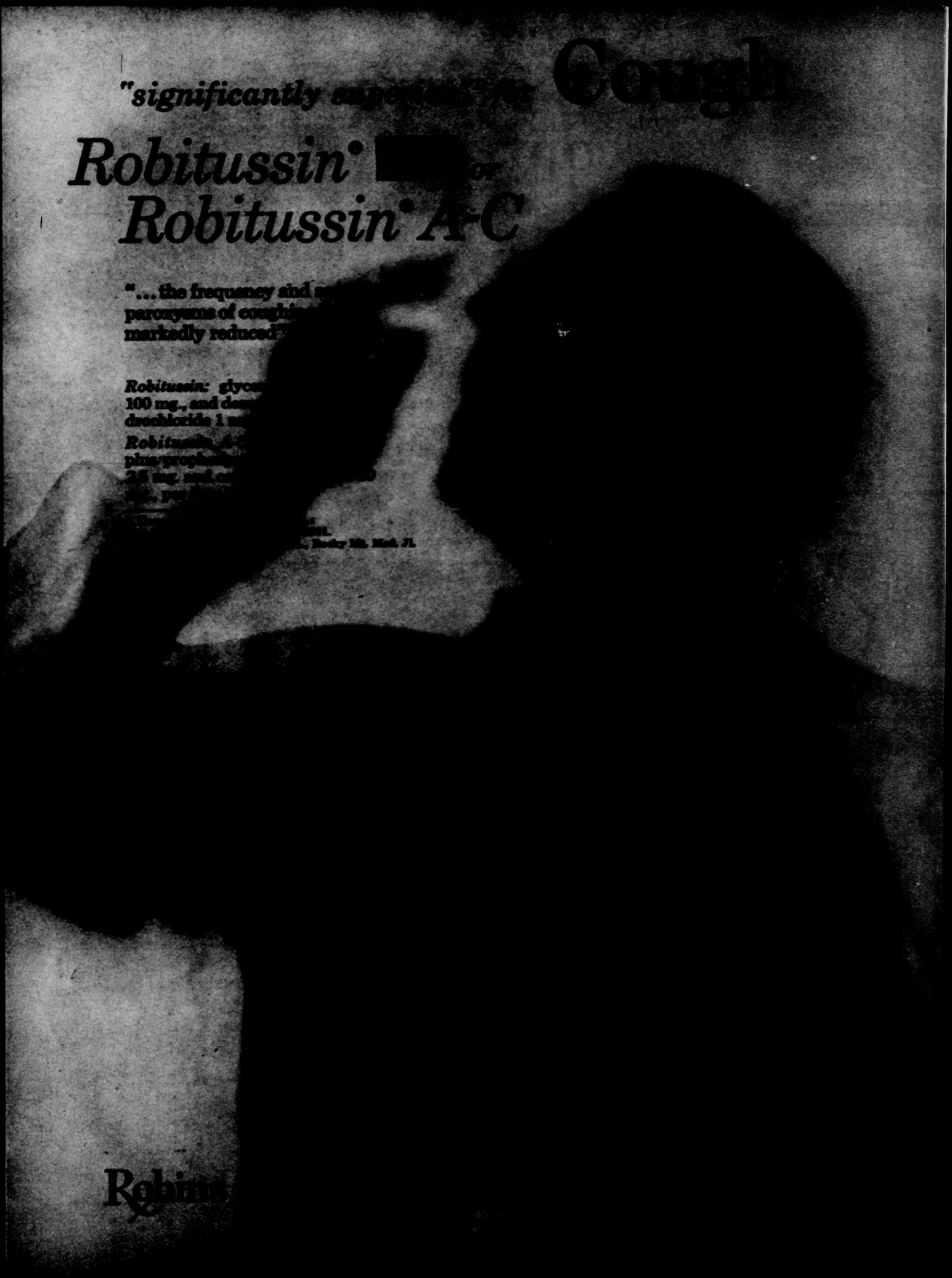
The film "CLINICAL ENZYMOLOGY" is now available for showing at medical meetings upon your request. And be sure to watch for the MED-AUDIOGRAPHS, a series of recorded clinical discussions.

REFERENCES
1. Dill, L. V., Med. Annals of D. C. 23:12, 1954
2. Greenblatt, R. B., Obst. & Gyn. 2:5, 1953
3. Javert, C. T., Obst. & Gyn. 3:4, 1954

PRODUCTS OF ORIGINAL RESEARCH

NATIONAL NATIONAL NATIONAL NATIONAL NATIONAL NATIONAL

THE NATIONAL DRUG COMPANY PHILADELPHIA 44, PA.


"significantly
Cough
Robitussin
Robitussin A-C
"... the frequency and
paroxysms of coughi
markedly reduced"
Robitussin: glyce
100 mg., and de
drochloride 1
Robitussin
plus propi
24 mg. and
Rob

more positive

diarrhea control

for all ages . . . in all seasons

Donnagel®

Robins

(Donnatal with Kaolin and Pectin Compound)

comprehensive correction of infectious, neuromuscular or emotional factors:

reduces hyperperistalsis
adsorbs toxins
soothes mucosa
neutralizes hyperacidity

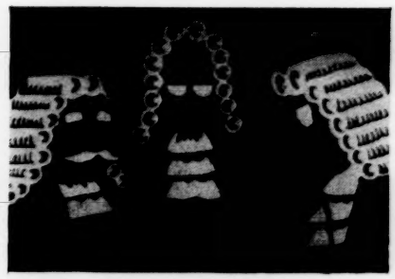
In each 30 cc:

Kaolin (90 gr.)	6.0 Gm.
Pectin (2 gr.)	130 mg.
Dihydroxy Aluminum Aminoacetate (7½ gr.)	0.5 Gm.
Hyoscyamine Sulfate	0.1037 mg.
Atropine Sulfate	0.0194 mg.
Hyoscine Hydrobromide	0.0065 mg.
Phenobarbital (¼ gr.)	16.2 mg.

Robins

A. H. ROBINS CO., INC.,
Richmond 20, Virginia

Ethical Pharmaceuticals of Merit since 1878



a "judicious combination..."

for antiarthritic therapy

SALCORT*

That cortisone and the salicylates have a complementary action has been well established.[1-5] In rheumatic conditions, functional improvement and a sense of feeling well are noted early. No withdrawal reactions have been reported.

One clinician states: "By a judicious combination of the two agents . . . it has been possible to bring about a much more favorable reaction in arthritis than with either alone. Salicylate potentiates the greatly reduced amount of cortisone present so that its full effect is brought out without evoking undesirable side reactions."[1]

INDICATIONS:

Rheumatoid arthritis . . . Rheumatoid spondylitis . . . Rheumatic fever . . . Bursitis . . . Still's disease . . . Neuromuscular affections

EACH TABLET CONTAINS:

Cortisone acetate	2.5 mg.
Sodium salicylate	0.3 Gm.
Aluminum hydroxide gel, dried .	0.12 Gm.
Calcium ascorbate	60 mg.
(equivalent to 50 mg. ascorbic acid)	
Calcium carbonate	60 mg.

* U.S. Pat. 2,691,662

1. Busse, E.A.: Treatment of Rheumatoid Arthritis by a Combination of Cortisone and Salicylates. Clinical Med. 11:1105 (Nov., 1955).
2. Roskam, J., VanCawenberge, H.: Abst. in J.A.M.A., 151:248 (1953).
3. Coventry, M.D.: Proc. Staff Meet., Mayo Clinic, 29:60 (1954).
4. Holt, K.S., et al.: Lancet, 2:1144 (1954).
5. Spies, T.D., et al.: J.A.M.A., 159:645 (Oct. 15, 1955).

BRISTOL, TENNESSEE
NEW YORK
KANSAS CITY
SAN FRANCISCO

The S. E. Massengill company

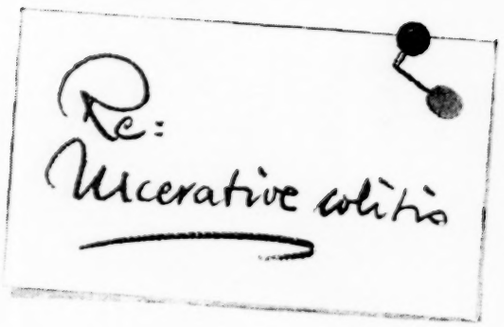
To counteract extremes of emotion

Desbutal®

(DESOXYN® *plus* NEMBUTAL®)

DESBUTAL gives the disturbed patient a new sense of well-being and energy, while calming his tensions and anxieties. One capsule represents 5 mg. DESOXYN Hydrochloride (Methamphetamine Hydrochloride, Abbott), and 30 mg. NEMBUTAL Sodium (Pentobarbital Sodium, Abbott). Bottles of 100 and 1000. Abbott

602100



This is "the most valuable drug that has been introduced for the treatment of ulcerative colitis" in recent years.[1] Results of treatment with Azulfidine "far exceed those of any previous drug used".[2] "It has been effective in controlling the disease in approximately two-thirds of patients who had previously failed to respond to standard colitis therapy currently in use."[3]

Azulfidine
BRAND OF SALICYLAZOSULFAPYRIDINE

1. BARGEN, J. A.: "Present Status of Hormonal and Drug Therapy of Ulcerative Colitis", South. M. J. *48*: 192 (Feb.) 1955.
2. BARGEN, J. A. and KENNEDY, R. L. J.: "Chronic Ulcerative Colitis in Children", Postgrad. Med. *17*: 127 (Feb.) 1955.
3. MORRISON, L. M.: "Response of Ulcerative Colitis to Therapy with Salicylazosulfapyridine", J. A. M. A. *151*: 366 (Jan. 31) 1953.

PHARMACIA LABORATORIES, INC.
270 Park Avenue, New York 17, N.Y.

microcytic anemia

one of the many anemias which can be effectively treated with

PERIHEMIN*

Hematinic *Lederle*

Nine out of 10 treatable anemias respond to PERIHEMIN. Its potent formula includes every known hemopoietic agent, including Purified Intrinsic Factor Concentrate. With this single product, you provide complete anemia therapy in a form convenient for the patient.

Dosage: one capsule, t.i.d.

Each capsule contains:

Vitamin B_{12} with Intrinsic Factor Concentrate	⅓ U.S.P. Oral Unit
Vitamin B_{12} (additional)	5 mcgm.
Ferrous Sulfate (Exsiccated)	192 mg.
Folic Acid	0.85 mg.
Ascorbic Acid (C)	50 mg.
Insoluble Liver Fraction	50 mg.

PERIHEMIN JR *Capsules*, for children, are approximately one-quarter the potency of this formula.

dry filled sealed capsules

(a Lederle exclusive!) for more rapid and complete absorption!

LEDERLE LABORATORIES DIVISION *AMERICAN Cyanamid COMPANY* PEARL RIVER, NEW YORK

*REG. U.S. PAT. OFF.

Just 1 small tablet daily helps meet the increased nutritional requirements of pregnancy...

ENGRAN

SQUIBB VITAMIN-MINERAL SUPPLEMENT

NEW FORMULA  NEW SMALL SIZE CAPSULE-SHAPED TABLET

just 1 small tablet daily supplies:

high supplemental dosages of the essential vitamins...

supplemental calcium— in phosphorus-free form...

supplemental iron...

trace elements...

for the support of a nutritionally perfect pregnancy

SQUIBB

Each Engran tablet supplies:

Vitamin A (synthetic)	5,000 U.S.P. Units
Vitamin D	500 U.S.P. Units
Thiamine mononitrate	3 mg.
Riboflavin	3 mg.
Pyridoxine HCl	2 mg.
Vitamin B_{12} activity concentrate	2 mcgm.
Folic acid	0.25 mg.
Niacinamide	20 mg.
Calcium pantothenate	5 mg.
Ascorbic acid	75 mg.
Calcium (as calcium carbonate)	150 mg.
Iron, elemental (as ferrous sulfate exsiccated)	10 mg.
Iodine (as potassium iodide)	0.15 mg.
Potassium (as the sulfate)	5 mg.
Cobalt (as the sulfate)	0.1 mg.
Copper (as the sulfate)	1 mg.
Magnesium (as the oxide)	6 mg.
Manganese (as the sulfate)	1 mg.
Zinc (as the sulfate)	1.5 mg.

Supplied in bottles of 100 and 1000 capsule-shaped tablets

it's a rare child* who doesn't

*

(and he's taking ABDEC Kapseals®)

PARKE, DAVIS & COMPANY · DETROIT, MICHIGAN


take to
50 CC.
ABDEC DROPS
THE NAME 'ABDEC' REG. U. S. PAT. OFF.
AQUEOUS VITAMIN SOLUTION
A non-alcoholic solution of vitamins, used for the prevention and treatment of vitamin deficiencies, particularly in children.
See carton for information.
Pat. No. 2,541,285
STOCK No. 22-1-68
PARKE, DAVIS & COMPANY
DETROIT, MICH., U.S.A.
or
1 PINT (475 cc.)
PALADAC
A PALATABLE NON-ALCOHOLIC AQUEOUS VITAMIN PREPARATION
EACH 4 CC. CONTAINS:
*Vitamin B12 Crystalline . . . . 5 mcg.
Vitamin C (Ascorbic Acid) 50 mg. (1⅔ M.D.R.)
Vitamin A 5000 units (1¼ M.D.R.)
Vitamin D 1000 units (2½ M.D.R.)
Vitamin B1 (Thiamine Hydrochloride) . . . 3 mg. (3 M.D.R.)
Vitamin B2 (Riboflavin) . . . 3 mg. (1½ M.D.R.)
Vitamin B6 (Pyridoxine Hydrochloride) . . . . . 1 mg.
Niacinamide (Nicotinamide) 20 mg.
*Pantothenic Acid (as the Calcium Salt) . . . . . 5 mg.
M.D.R. = Minimum daily requirement for adults.
*Used in human nutrition has not been established.
A vitamin supplement particularly adapted for prevention of vitamin deficiencies in infants and children. Also suitable for adults.
DOSE — Infants, 1/2 teaspoonful (2 cc.) daily; children and adults, 1 teaspoonful (4 cc.) daily; or more as directed by the physician.
If desired, may be administered in milk or fruit juice, or other foods as preferred.
SHAKE WELL BEFORE USING
REFRIGERATION NOT REQUIRED
Dispense unbroken package only.
U. S. Patent 2541285
P188M
Stock 22-211-48
PARKE, DAVIS & CO.
DETROIT, MICH., U.S.A.

PHOTO DATA. CAMERA: 4X5 REFLEX; EXPOSURE: 1/200 SEC. AT F.8 EXISTING LIGHTING.

ACHROMYCIN*

Tetracycline *Lederle*

widely prescribed because of these important advantages:

1) rapid diffusion and penetration
2) prompt control of infection
3) true broad-spectrum activity (proved effective against a wide variety of infections caused by Gram-positive and Gram-negative bacteria, rickettsiae, and certain viruses and protozoa)
4) negligible side effects
5) every gram produced in Lederle's *own* laboratories under rigid quality control, and offered *only* under the Lederle label
6) a *complete* line of dosage forms

in prolonged illness, prescribe

ACHROMYCIN SF

TETRACYCLINE with STRESS FORMULA VITAMINS

Attacks the infection, bolsters the body's natural defense. Stress vitamin formula suggested by the National Research Council in *dry-filled, sealed* capsules with ACHROMYCIN, 250 mg. Also available: ACHROMYCIN SF ORAL SUSPENSION (Cherry Flavor), 125 mg. per 5 cc.

dry-filled sealed capsules

(a Lederle exclusive!) for more rapid and complete absorption. No oils, no paste, tamperproof!

LEDERLE LABORATORIES DIVISION *AMERICAN Cyanamid COMPANY* PEARL RIVER, NEW YORK

Lederle

*REG. U. S. PAT. OFF.

THE COUGH STRUCK ONE...

Hickory, dickory, dock,
The cough went round the clock.

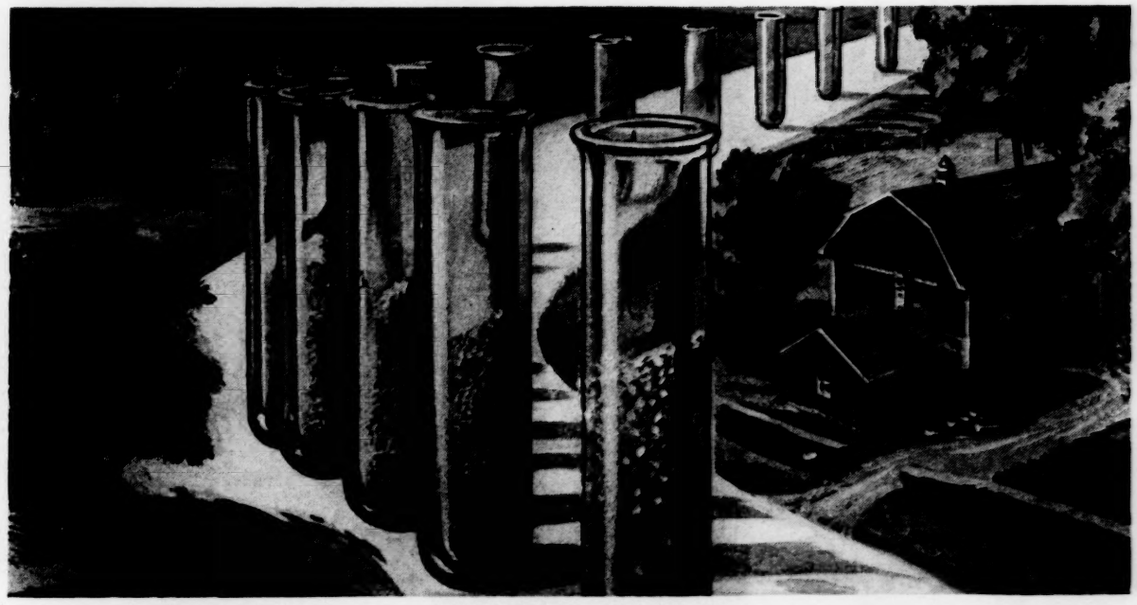
PHENERGAN Expectorant quiets cough and calms the patient through *four* distinct actions—topical anesthetic, antihistaminic, sedative, and expectorant. It is the round-the-clock preference of many clinicians, their agent of choice in cough control.

PHENERGAN®
EXPECTORANT

Promethazine Expectorant with Codeine
Plain (without Codeine)

Wyeth®
Philadelphia 1, Pa.

SECOND REPORT



LECITHIN RESEARCH—AT THE BEND OF THE ROAD

The Therapeutic Usefulness of Lecithin – a natural phospholipid

Because lecithin, a natural, edible food constituent, is an excellent emulsifying agent its application in diseases characterized by disturbed fat absorption and metabolism is logical. Research has proved its value in facilitating intestinal absorption of fats and fat-soluble substances such as Vitamin A.[1-5] For this reason it suggests itself as worthy of trial in treating underweight and steatorrheal diseases (sprue, celiac disease, etc.).

Encouraging results were also achieved in the management of psoriasis, together with dietary and topical measures,[6] and in fatty livers.[7] In the treatment of diabetes, lecithin together with vitamin E has reduced insulin requirements in certain patients.[8] Research on its potentially useful role in the management of the more complicated forms of deranged lipid and cholesterol metabolism – as encountered in essential hyperlipemia, idiopathic familial hypercholesteremia, xanthomatosis, diabetes, etc. – is now being actively conducted.

An excellent source of lecithin is Glidden's "RG" Oil-free Soya Lecithin, a highly purified extract containing a minimum of 95% phospholipids. It is packed in a specially designed 8 oz container to maintain its purity and freshness and is available at your drugstore.

Dosage: Investigators of lecithin have used quantities from 7.5 to 30 grams daily in divided doses. (3 teaspoonfuls equal 7.5 grams.)

Administration: "RG" Lecithin is presented in palatable granules which may be taken plain, in milk, in orange juice or other citrus juices, or sprinkled on cereal.

Literature available on request.

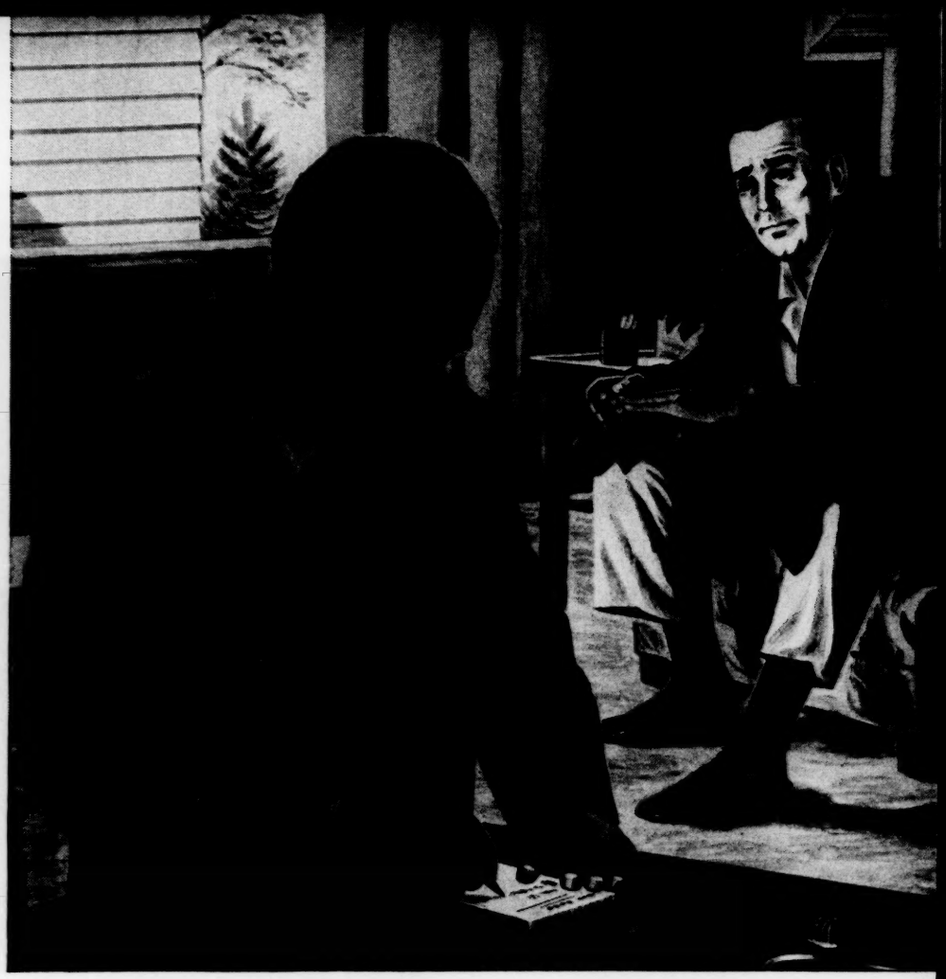
Bibliography: 1. Adlersberg, D., and Sobotka, H.: J. Nutrition *25*:255 (March) 1943. • 2. Adlersberg, D., and others: Gastroenterology *10*:822 (May) 1948. • 3. Adlersberg, D.: New York J. Med. *44*:606 (March 15) 1944. • 4. Adlersberg, D., and others: Am. J. Digest. Dis. *16*:333 (Sept.) 1949. • 5. Augur, V.; Rollman, H. S., and Deuel, H. J., Jr.: J. Nutrition *33*:177 (Feb.) 1947. • 6. Gross, P., and Kesten, M. B.: New York J. Med. *50*:2683 (Nov. 15) 1950. • 7. Schettler, G.: Klin. Wchnschr. *30*:627 (July) 1952. • 8. Dietrich, H. W.: South. M. J. *43*:743 (Aug.) 1950.

GLIDDEN RG® LECITHIN

THE GLIDDEN COMPANY • CHEMURGY DIVISION
1825 North Laramie Avenue, Chicago 39, Illinois

QUALITY Glidden PRODUCTS

BACK ON HIS FEET BUT STILL SICK...



The Problem of Residual Anemia in Upper Respiratory and Other Infections

The persistent anemia which you so frequently see in association with an infectious process demands serious consideration since it "favours the development of further infection and may retard convalescence."[1]

Noteworthy is the slow recovery of the anemic patient following viral or bacterial upper respiratory involvement.

Cobalt appears to be the only known agent capable of stimulating the depressed bone-marrow function typical of post-infection anemia.

RONCOVITE® presents the original, clinically proved, pure cobalt-iron product. Thorough investigation has verified the effectiveness and safety of Roncovite.

Continuing Proof of Roncovite's Effectiveness In Anemia Associated with Infection

"Cobalt appears to be a valuable drug in the treatment of anemias secondary to chronic diseases."[2]

"The marked increase in the early erythroid cells in the [children] . . . with anaemia of infection point to a direct stimulation of the erythroid tissue of the marrow as the main action of the cobalt."[1]

". . . [cobalt] will force the bone marrow to make more cells even when nephritis or chronic infection are the causes of the anemia."[3]

"There is no doubt that given in sufficient dosage . . . [cobalt] is effective in alleviating the anemia secondary to the infection, cancer, and renal disease."[4]

". . . cobalt appeared to be a useful and valuable drug, well tolerated and devoid of undue toxicity."[2]

RONCOVITE®

SUPPLIED:

Roncovite Tablets—red, enteric coated in bottles of 100. Roncovite-OB—red, capsule-shaped tablets in bottles of 100. Roncovite Drops — bottles of 15 cc. with calibrated dropper.

DOSAGE:

One tablet after each meal and at bedtime. Children, 1 year or over, 0.6 cc. (10 drops); infants less than 1 year, 0.3 cc. (5 drops) once daily diluted with water, milk, fruit or vegetable juice.

REFERENCES:

1. Coles, B. L.: Arch. Disease in Childhood *30*:121 (April) 1955.
2. Weinsaft, P. P., and Bernstein, L. H. T.: Amer. J. Med. Sc. *230*:264 (Sept.) 1955.
3. Vilter, R. W.: Amer. J. Clin. Nutr. *3*:72 (Jan.-Feb.) 1955.
4. Cartwright, G. E.: Amer. J. Clin Nutr. *3*:11 (Jan.-Feb.) 1955.

LLOYD BROTHERS, INC. *Cincinnati 3, Ohio*
In the Service of Medicine Since 1870

THERAPEUTIC
FORMULA
multivitamins

each small *OPTILET contains:*

Vitamin A	7.5 mg. (25,000 units)
Vitamin D	25 mcg. (1000 units)
Thiamine Mononitrate	10 mg.
Riboflavin	5 mg.
Nicotinamide	150 mg.
Vitamin B_{12}	6 mcg.
Ascorbic Acid	150 mg.

Abbott

602126

HydroCortone® -T B A

(HYDROCORTISONE TERTIARY-BUTYLACETATE, MERCK)

gives the arthritic patient more days of freedom from joint symptoms—in many patients the anti-rheumatic effect persists 2 to 10 times longer than after injection of hydrocortisone acetate. Its action is local and without systemic effect.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.

SUPPLIED: SALINE SUSPENSION HYDROCORTONE-TBA—25 MG./CC., VIALS OF 5 CC.

LIVITAMIN® with IRON
each fluidounce contains:

Iron peptonized.....420 mg.
(Equiv. in elemental iron to 70 mg.)
Manganese citrate, soluble.....158 mg.
Thiamine hydrochloride..... 10 mg.
Riboflavin..... 10 mg.
Vitamin B_{12} (crystalline) ..... 20 mcg.
Niacinamide..... 50 mg.
Pyridoxine hydrochloride..... 1 mg.
Pantothenic acid ..... 5 mg.
Liver fraction 1..... 2 Gm.
Rice bran extract..... 1 Gm.
Inositol ..... 30 mg.
Choline ..... 60 mg.

. . . the reconstructive iron tonic of wide application . . .

LIVITAMIN®

WITH IRON

In debilitation, syndrome therapy instead of symptom treatment is required. Livitamin (Massengill) provides comprehensive therapy and adequate nutritional support. The appetite improves, as does the blood picture . . . improved anabolism and better digestion produce a significant syndrome reversal.

LIVITAMIN® CAPSULES with INTRINSIC FACTOR
each capsule contains:

Desiccated liver.....450 mg.
Ferrous sulfate.....130 mg.
(Equiv. to 25 mg. of elemental iron)
Thiamine hydrochloride..... 3 mg.
Riboflavin..... 3 mg.
Niacinamide..... 10 mg.
Vitamin B_{12} ..... 5 mcg.
Pyridoxine hydrochloride.....0.5 mg.
Calcium pantothenate..... 2 mg.
Folic acid..... 1 mg.
Intrinsic factor USP.....1/6 Unit

. . . in pernicious anemia and geriatrics . . .

LIVITAMIN®

CAPSULES WITH INTRINSIC FACTOR

Intrinsic factor is essential to provide full utilization of antianemic and nutritional factors in P. A. and many Geriatric patients. Livitamin Capsules with Intrinsic Factor (Massengill) contain intrinsic factor, U.S.P., iron and the B-complex vitamins. This integrated medication provides an optimal response in these difficult patients.

THE S. E. MASSENGILL COMPANY
BRISTOL, TENNESSEE

For an Estimated 4 out of 1,000,000 People More Than 3 Years of Pharmaceutical Research

In 1855 Addison first delineated the classical clinical picture of adrenocortical insufficiency. Today, as then, Addison's disease is a rare (an estimated 4 per 1,000,000 population) but serious affliction.

The synthesis of desoxycorticosterone was an important step in the control of adrenocortical insufficiency. However, the easy solubility and consequent short activity of this potent hormone steroid imposed severe limitations to its use.

CIBA, constantly in the forefront of endocrine research, spent more than 3 years developing a desoxycorticosterone derivative that would be more slowly absorbed and provide more prolonged activity. The result was **Percorten trimethylacetate.**

A single intramuscular injection of **Percorten trimethylacetate** maintains the patient with adrenal insufficiency for about a month. With this new long-acting hormone, the unpredictable and inconvenient process of surgical implantation of desoxycorticosterone pellets is no longer necessary.

The introduction of **Percorten trimethylacetate** marks a significant advance in therapy of adrenocortical insufficiency. Its development is typical of CIBA research—the persistent, painstaking quest for better pharmaceutical specialties, whether for the rare or the widespread diseases.

Supplied: Multiple-dose Vials, 4 ml., containing 25 mg. per ml. as an aqueous microcrystalline suspension for intramuscular use only.

Percorten® trimethylacetate (desoxycorticosterone trimethylacetate CIBA)

C I B A
SUMMIT, N. J.

2/2008M

REPRINT ORDER FORM

THE AMERICAN JOURNAL OF MEDICINE, 49 W. 45th St., New York 36, N. Y.

Please send me the following Seminars reprinted from the AMERICAN JOURNAL OF MEDICINE:

☐ GASTROINTESTINAL PHYSIOLOGY	$2.00
☐ BLOOD COAGULATION	$2.00
☐ ANTI HYPERTENSIVE DRUGS	$2.00
☐ HEMOLYTIC ANEMIAS	$2.00

Enclosed is my check

NAME_____

ADDRESS_____

CITY_____ STATE_____

NEW *for those with* PARKINSONISM

Smoother activity

and

brighter expression

with 'KEMADRIN'®*

- reduces rigidity and tremor.
- seldom causes dryness of the mouth, blurring of vision or excitation.

***'KEMADRIN'** brand Procyclidine Hydrochloride Tablet of 5 mg., scored. Bottles of 100 and 1,000.

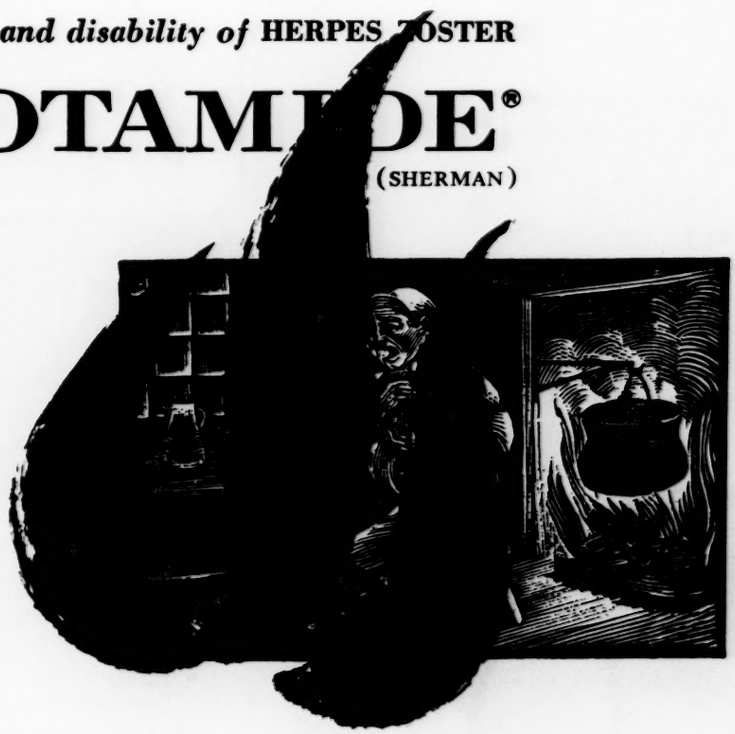
Literature available on request.

BURROUGHS WELLCOME & CO. (U. S. A.) INC., Tuckahoe, N.Y.

for the pain and disability of **HERPES ZOSTER**

PROTAMIDE®

(SHERMAN)



published studies show:*

Improvement is "almost immediate," with "good to excellent results" in four out of five patients, and no postherpetic neuralgia in any patient who responded favorably.

Protamide is a sterile colloidal solution prepared from animal gastric mucosa . . . denatured to eliminate protein reaction . . . completely safe and virtually painless by intramuscular injection.

Clinical data on request.

use* PROTAMIDE® *first

in herpes zoster and post-infection neuritis

SHERMAN LABORATORIES
BIOLOGICALS & PHARMACEUTICALS

* Combes, F. C. & Canizares, O.: New York St. J. Med. 52:706, 1952; Marsh, W. C.: U. S. Armed Forces M. J. 1:1045, 1950.

to speed defervescence to speed convalescence

PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
Brooklyn 6, N. Y.

more than 42,000,000 doses of ACTH have been given

HP*ACTHAR *Gel*

Unsurpassed in safety and efficacy

In a series of patients treated continuously with Armour ACTH for at least 5½ years[1] . . .

- Each responded with a maintained increase in cortical function
- Major and minor surgical and obstetrical procedures caused no incidents
- Sudden discontinuance of ACTH did not provoke a crisis

. . . and HP*ACTHAR *Gel* should be used routinely to minimize adrenal suppression and atrophy in patients treated with prednisone, prednisolone, hydrocortisone and cortisone.

HP*ACTHAR *Gel* is the most widely used ACTH preparation

*Highly purified

1. Wolfson, W. Q.: Mississippi Valley M. J. 77: 66, 1955.

THE ARMOUR LABORATORIES
A DIVISION OF ARMOUR AND COMPANY • KANKAKEE, ILLINOIS

new...paired piperidol action
for functional gastrointestinal complaints

rapid
visceral eutonic, Dactil®

prolonged
cholinolytic, Piptal®

relief throughout the G. I. tract

Tridal

TRIDAL permits more comprehensive control of gastrointestinal complaints by providing the combined benefits of two piperidols. The local action of Dactil* works immediately to give **rapid** relief of gastrointestinal pain and spasm; the potent cholinolytic Piptal† reinforces relief and provides **prolonged** normalization of secretion and motility.

TRIDAL is singularly free from urinary retention, constipation, dry mouth, blurred vision.

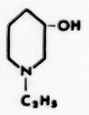
dosage: One TRIDAL Tablet two or three times a day and at bedtime. Unless rapidly swallowed with water, TRIDAL will produce some lingual anesthesia.

Each TRIDAL Tablet contains 50 mg. of Dactil and 5 mg. of Piptal. Bottles of 50 compressed, uncoated tablets.

*Dactil (the ***only*** brand of N-ethyl-3-piperidyl diphenylacetate hydrochloride): the piperidol to prescribe alone when no interference with digestive secretion is desired.

†Piptal (the ***only*** brand of N-ethyl-3-piperidyl-benzilate methobromide): the piperidol to prescribe alone when peptic ulcer is known to be present and normalization of secretion as well as motility is desired.

Lakeside Laboratories, Inc. PIONEERS IN PIPERIDOLS MILWAUKEE 1, WISCONSIN



93185

To Brighten the Diet . . .

. . . to make days and nights more pleasant for the aged patient

An appetite stimulant . . . mild euphoretic . . . appealing sedative at bedtime . . . a supplemental natural source of minerals, vitamins, and readily absorbable nutriments—these are some of the roles that wine can play in the daily diet of your aged or convalescent patient.

Few substances—natural or artificial—can offer the unique combination of qualities found in wine, the traditional beverage of moderation. Praised through the ages for its "tonic" effect, wine has been intensively studied since 1939 by American laboratory and clinical investigators. These modern tests have revealed the physiological basis for subjective theories of past years, and are now explaining the action and fate of wine and its components in the body.

Many of the important physiological properties of wine differ significantly from those of plain alcohol. Wine increases appetite and heightens olfactory acuity. It stimulates the flow of salivary juices. Buffered by its own natural salts and organic acids, it provides a mild, prolonged stimulation of gastric secretion. This same buffer effect makes the diuresis produced by wine a slow, moderate one.

Wine is also a ready and pleasant source of nutrient energy, and of absorbable iron and other essential minerals. The vasodilating action of wine aids toward improving circulation and increasing cardiac output.

A bit of sherry or light wine before meals, table wine with luncheon or dinner, or a glass of port at bedtime can add a welcome touch of interest and "elegance" to the daily routine of the convalescent and the elderly patient. The day seems shorter and brighter, and the night more pleasant and relaxed.

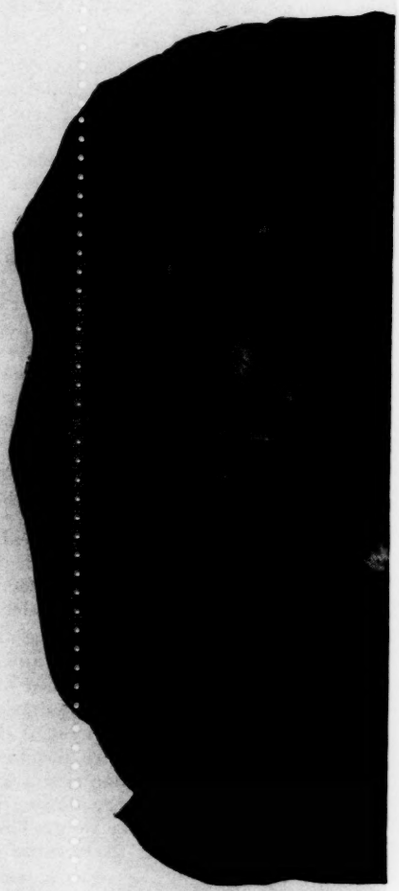
For a few cents a day your patients can have wines produced from the world's finest grape varieties, grown in an ideal climate and handled with consummate skill. Research information on wine is available upon request. Wine Advisory Board, San Francisco 3, California.

MAXIMUM SAFE ANALGESIA

in whatever potency each patient may require

By facilitating the optimal analgesic medication of each patient, PHENAPHEN and PHENAPHEN WITH CODEINE have proven their wide range of clinical usefulness — for cases of simple headache to many of late cancer.

True pharmacodynamic synergism enhances the therapeutic potency of each of the 4 forms available for discriminating prescription:



PHENAPHEN
— *basic non-narcotic formula*
Each brown and white capsule contains:
Acetylsalicylic acid (2½ gr.).....162 mg.
Phenacetin (3 gr.).....194 mg.
Phenobarbital (¼ gr.).....16.2 mg.
Hyoscyamine sulfate ($\frac{1}{2000}$ gr.)..0.031 mg.

Phenaphen No. 2
PHENAPHEN
with CODEINE PHOSPHATE ¼ GR.
Each black and yellow capsule contains:
The basic phenaphen formula plus
Codeine phosphate (¼ gr.).....16.2 mg.

Phenaphen No. 3
PHENAPHEN
with CODEINE PHOSPHATE ½ GR.
Each black and green capsule contains:
The basic phenaphen formula plus
Codeine phosphate (½ gr.).....32.4 mg.

Phenaphen No. 4
PHENAPHEN
with CODEINE PHOSPHATE 1 GR.
Each green and white capsule contains:
The basic phenaphen formula plus
Codeine phosphate (1 gr.).....64.8 mg.

A. H. ROBINS CO., INC. • Richmond 20, Virginia
Ethical Pharmaceuticals of Merit since 1878

Phenaphen® Robins
Phenaphen® with Codeine

Cremomycin.®

SULFASUXIDINE® — NEOMYCIN SUSPENSION WITH PECTIN AND KAOLIN

new — for prompt diarrhea control, quick return to work

MAJOR ADVANTAGES: Combines 'Sulfasuxidine' with neomycin for broader antibacterial action. Pectin and kaolin have soothing, detoxifying properties. Fruit flavor tastes good.

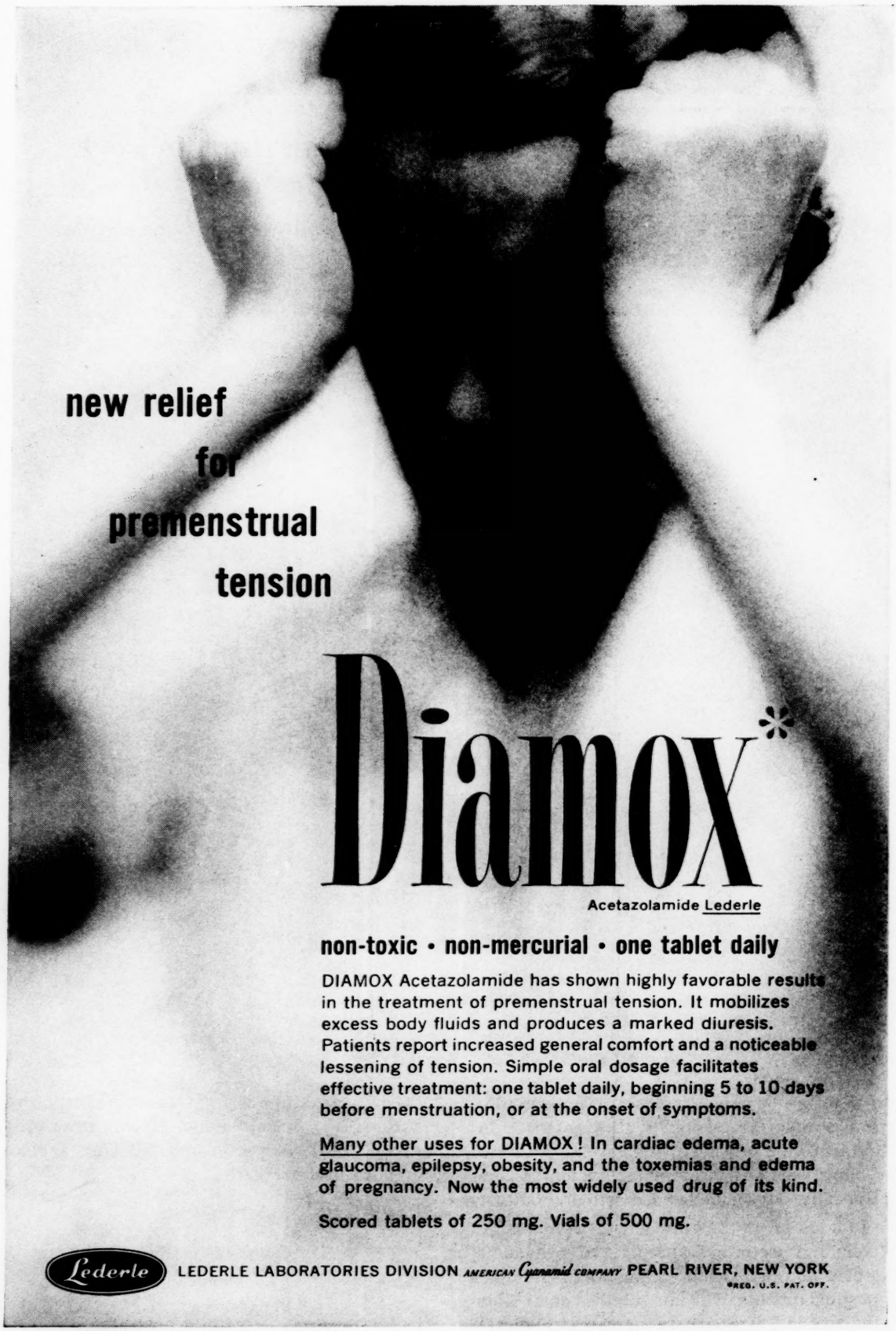
In diarrhea—whether specific or nonspecific—prompt relief is assured with CREMOMYCIN. 'Sulfasuxidine' and neomycin have a wide range of effectiveness—their combined action is complete and prompt, and they are virtually nontoxic.

In addition to the antibacterial components, kaolin and pectin in CREMOMYCIN provide adsorbent and detoxicant action, soothe inflamed intestinal mucosa. The fine subdivision of all ingredients in CREMOMYCIN increases its efficacy.

Each fl. oz. (30 cc.) of CREMOMYCIN contains 3.0 *Gm.* 'Sulfasuxidine,' 300 *mg.* neomycin sulfate, 0.3 *Gm.* pectin and 3.0 *Gm.* kaolin. Supplied in 8 oz. bottles.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.


new relief
for
premenstrual
tension
Diamox*
Acetazolamide Lederle
non-toxic • non-mercurial • one tablet daily
DIAMOX Acetazolamide has shown highly favorable results in the treatment of premenstrual tension. It mobilizes excess body fluids and produces a marked diuresis. Patients report increased general comfort and a noticeable lessening of tension. Simple oral dosage facilitates effective treatment: one tablet daily, beginning 5 to 10 days before menstruation, or at the onset of symptoms.
Many other uses for DIAMOX! In cardiac edema, acute glaucoma, epilepsy, obesity, and the toxemias and edema of pregnancy. Now the most widely used drug of its kind.
Scored tablets of 250 mg. Vials of 500 mg.
Lederle
LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY PEARL RIVER, NEW YORK
*REG. U.S. PAT. OFF.

Upjohn

Relax
the nervous,
tense,
emotionally unstable:

Reserpoid *(Pure crystalline alkaloid)*

TRADEMARK FOR THE UPJOHN BRAND OF RESERPINE

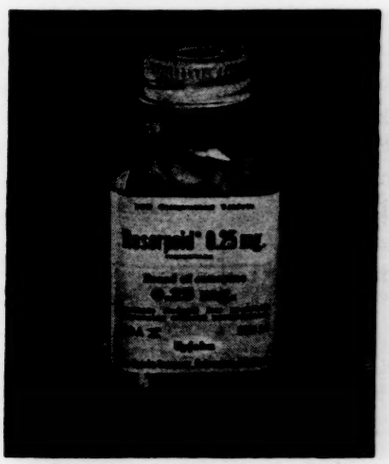
Each tablet contains:

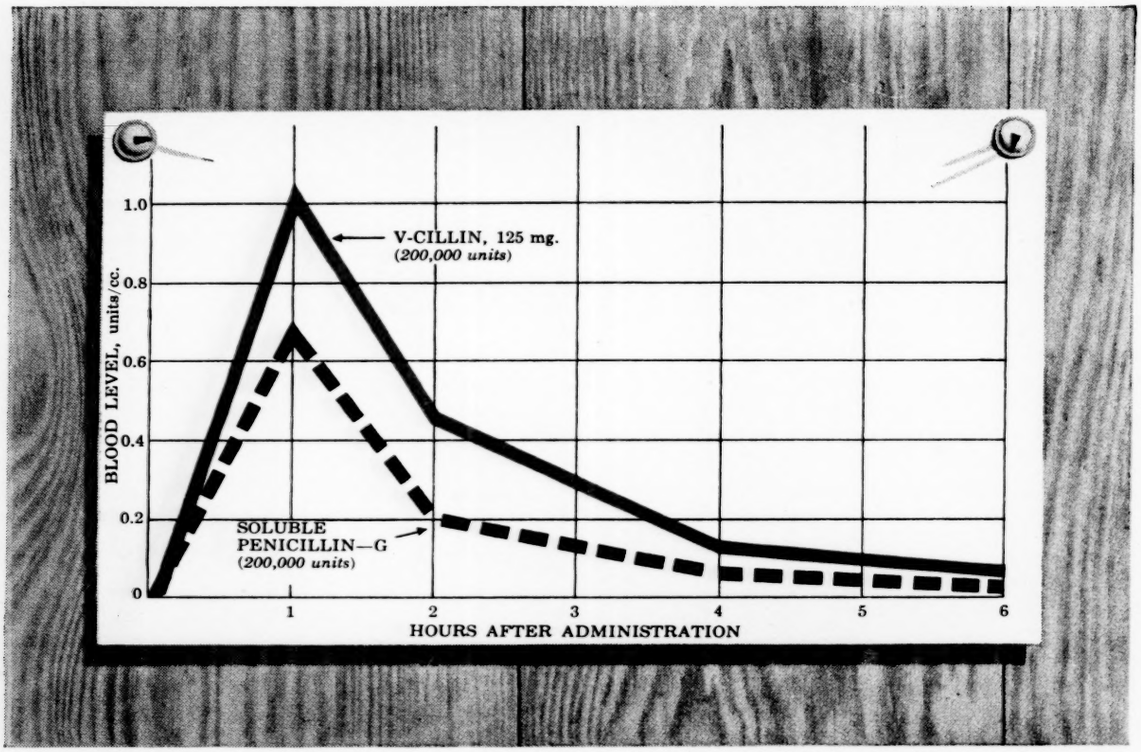
Reserpine . . . . . 0.1 mg.
or 0.25 mg.
or 1.0 mg.

Supplied:

Scored tablets
0.1 and 0.25 mg. in bottles of 100 and 500
1.0 mg. in bottles of 100

The Upjohn Company, Kalamazoo, Michigan





new superior oral penicillin

V-CILLIN

(PENICILLIN V, LILLY)

'V-Cillin' was developed by the Lilly Research Laboratories to fulfill the need for an acid-resistant penicillin—for a more dependable and effective oral penicillin.

Gastric acidity does not significantly affect the potency of 'V-Cillin' ('V-Cillin' is an acid). In contrast, 50 percent of the potency of potassium penicillin G may be destroyed by gastric acids, in ten to thirty minutes. Thus, 'V-Cillin' eliminates a major variable in oral penicillin therapy, produces 50 to 100 percent higher blood levels, and makes the oral use of penicillin much more feasible.

In the duodenum, absorption of 'V-Cillin' begins immediately.

DOSAGE: 125 or 250 mg. t.i.d. May be administered without regard to mealtimes.

SUPPLIED: As attractive green-and-gray pulvules of 125 mg. (200,000 units), in bottles of 50. Also, pediatric suspension providing 125 mg. per 5-cc. teaspoonful, in bottles of 80 cc.

Lilly

QUALITY / RESEARCH / INTEGRITY

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U.S.A.

630029

The American Journal of Medicine

VOL. XX FEBRUARY, 1956 No. 2

Editorial

Molecular Events in Cardiac Failure

THE clinical diagnosis of congestive heart failure is made by eliciting a well-recognized constellation of signs and symptoms. Advances in the understanding of congestive heart failure as a clinical entity, however, have been due largely to intensive physiologic investigation of the altered hemodynamics and the altered function of the heart and of many other viscera in this pathologic state. In recent years, as a matter of fact, clinical investigators have become so engrossed in the study of organs other than the heart in congestive heart failure that the facetious statement attributed to the late Henry Christian might be recalled to illustrate the irony of the situation. Dr. Christian is quoted as summarizing a symposium at the Harvard Medical School dealing with the pathogenesis of congestive heart failure by saying, "Gentlemen, I tell you, in conclusion, that the heart has nothing whatsoever to do with the syndrome of congestive heart failure!"

Nothing, of course, could be further from the truth. The interplay of primary and secondary factors in the genesis of the full-blown clinical syndrome is subtle, intricate and controversial, although there is general agreement that in congestive heart failure the heart fails to pump blood at a rate commensurate with the needs of the tissues. This latter definition of congestive heart failure, of course, does not tell us what is happening in the myocardium. Recent investigations of myocardial metabolism in normal persons and in those with cardiac failure have thrown some light upon this question, although a great deal remains to be learned.

The classic studies of Starling[1] on the behavior of the heart-lung preparation under different conditions of venous supply and peripheral resistance have given rise to a generalization known as Starling's Law of the Heart. This Law states that the energy of contraction of the myocardium is a function of the length of its muscle fibers. Plots of stroke work versus diastolic fiber length (or end-diastolic filling pressure) in the heart-lung preparation showed that "normally" hearts responded to increasing filling pressures with increased stroke outputs up to a point beyond which further increases in filling pressure and diastolic fiber length produced decreases in stroke work (the "down-limb" of the Starling curve). Under these circumstances, however, the oxygen consumption of the heart increased linearly with diastolic volume over a wide range of fiber lengths including those at which cardiac work began to decrease.[2] Failure of the heart was associated with the descending limb of the Starling curve which represented an unphysiologic relationship between diastolic fiber length and stroke work.

Recently this concept has been refined by Sarnoff and his collaborators[3] working with the open-chest dog. This group found that in such a preparation there is no descending limb to ventricular function curves (Starling curves) in the normal heart, although a plateau is reached at high filling pressures. Further they found that

[1] STARLING, E. H. Linacre Lecture on the Law of the Heart. Cambridge, 1915; New York, 1918. Longmans, Green & Co.

[2] EVANS, C. L. and MATSUOKA, Y. The effect of various mechanical conditions upon the gaseous metabolism and efficiency of the mammalian heart. *J. Physiol.*, 49: 578, 1915.

[3] SARNOFF, S. J. Myocardial contractility as described by ventricular function curves; observations on Starling's law of the heart. *Physiol. Rev.*, 35: 107, 1955.

any one heart may be characterized by a family of ventricular function curves representing differing states of cardiac competence, and that the descending limb occurs only after a shift from a normal to an abnormal ventricular function curve, the latter being induced by producing anemia, coronary stenosis or valvular insufficiency. In terms of the Starling concept, then, as extended by Sarnoff, cardiac failure can be defined as a change in the condition of the myocardium which shifts performance from a normal ventricular function curve to a depressed one. Barger, Roe and Richardson[4] have made similar observations in dogs with experimental valvular disease and Dexter and his colleagues[5] have obtained a family of ventricular function curves in humans with various degrees of cardiac competence. An abnormal relationship between end-diastolic filling pressure and stroke work for a given ventricle may thus be one functional definition of cardiac failure.

Since, however, the ability of the heart to do work depends basically upon the biochemical activity which leads to muscular contraction, it would appear that the final definitive description of cardiac failure will be made in terms of the enzymatic and other biochemical changes which occur in the failing myocardial cell. Cardiac work can be performed at optimum rates only when all the reactions concerned with the oxidation of substrate, generation of high energy phosphate bonds and utilization of this bond energy in the final contractile process (the shortening of actomyosin)[6] are carried out at satisfactory rates. Interruption of this sequence of biochemical events at any stage, therefore, could lead to cardiac failure.

Several years ago Olson and Schwartz[7] analyzed data available from studies of cardiac metabolism in isolated tissue preparations, perfused hearts and intact animals and man. They proposed a tentative classification of certain types of congestive heart failure on the basis of specific disturbances in myocardial metabolism. Work carried out in this and in other laboratories[8] during the interim has generally supported this hypothesis.

According to this analysis, the high output cardiac failure which may accompany anemia, beriberi and thyrotoxicosis is caused by defects in energy production by the heart, that is, in enzymatic processes concerned with oxidation of substrate and/or oxidative phosphorylation leading to the formation of adenosine triphosphate (ATP) or creatine phosphate (CP). The metabolic defect in this type of failure appears to be generalized and to involve tissues other than the heart. As a result of this, vasodilatation occurs, the cardiac output rises until the work capacity of the heart, lowered by the metabolic defect, is exceeded and failure occurs. It is now apparent that cholemia and uremia may also produce sufficiently serious disturbances in energy production to cause high output failure in man.

The more common type of low output failure seen in patients with hypertension, coronary artery disease and valvular disease, on the other hand, appears to be due to failure in the energy utilization of the heart, that is, the transformation of high energy phosphate bonds into contractile work. Study of patients with this type of congestive heart failure by coronary sinus catheterization by Bing et al.[9] and by Goodale et al.[10] has failed to reveal any defect in the uptake of substrate or oxygen by the failing heart. Further, study of the high energy phosphate compounds ATP and CP in the myocardium of the failing heart-lung preparation by Wollenberger[11] and in the myocardium of the dog in experimental low output failure due to valvular disease by Olson[12] has demonstrated no depletion of these sources of free energy for

[4] Barger, A. C., Roe, B. B. and Richardson, G. S. Relation of valvular lesions and of exercise to auricular pressure, work tolerance, and to development of chronic congestive failure in dogs. *Am. J. Physiol.*, 169: 384, 1952.

[5] Dexter, L., Lewis, B. M., Houssay, H. E. J. and Haynes, F. W. The dynamics of both right and left ventricles at rest and during exercise in patients with heart failure. *Tr. A. Am. Physicians*, 66: 266, 1953.

[6] Szent-Gyorgyi, A. The Chemistry of Muscular Contraction. New York, 1948. Academic Press.

[7] Olson, R. E. and Schwartz, W. B. Myocardial metabolism in congestive heart failure. *Medicine*, 30: 21, 1951.

[8] Bing, R. J. Myocardial metabolism. *Circulation*, 12: 635, 1955.

[9] Bing, R. J., Siegel, A., Vitale, A., Balboni, F., Sparks, E., Taeschler, M., Klapper, M. and Edwards, S. Metabolic studies of the human heart *in vivo*. I. Studies of carbohydrate metabolism of the human heart. *Am. J. Med.*, 15: 284, 1953.

[10] Goodale, W. T., Olson, R. E. and Hackel, D. B. Myocardial glucose, lactate and pyruvate metabolism of normal and failing hearts studied by coronary venous catheterization in man. *Federation Proc.*, 9: 49, 1950.

[11] Wollenberger, A. On the energy-rich phosphate supply of the failing heart. *Am. J. Physiol.*, 150: 733, 1947.

[12] Olson, R. E. Symposium on cardiac metabolism. American Physiology Society, New York, 1952.

contraction. The evidence favors the view that in low output failure the biochemical defect lies in the assimilation of phosphate-bond energy by the contractile proteins or in the contractile proteins themselves.

In order to clarify this point a comprehensive study of the properties of the contractile proteins of heart muscle from normal dogs and from dogs in congestive heart failure due to valvular disease was undertaken in our laboratory some time ago. A study of the physical-chemical properties of myosin isolated from the normal heart of the dog led to the conclusion that the molecular weight of normal cardiac myosin is considerably lower[13] than that reported for rabbit skeletal myosin.[14] It has been found, further, that in heart failure in the dog there are marked changes in the physical-chemical properties of cardiac myosin consistent with a sizeable increase in molecular weight.[15] The properties of cardiac actomyosin, likewise, appear to be altered by chronic congestive heart failure in the dog.[16]

It seems clear that the properties of myosin may change *in vivo*. The concept of a "native protein" as representing a single, fixed intracellular entity is probably a great oversimplification. In support of the view that myosin may be subject to such changes is the variability of the physical data on rabbit skeletal myosin isolated in different laboratories,[14] the formation of identical lower molecular weight fragments from skeletal myosin (heavy and light meromyosins) by treatment with trypsin[17] and chymotrypsin,[18] and the action of urea and guanidine salts in depolymerizing skeletal myosin.[19] Parallel studies of cardiac myosin isolated from normal and failing hearts are sorely needed.

The finding of changes in the physical-chemical properties of cardiac myosin in heart failure suggests that the physical-chemical state of a protein may affect its biologic activity. It has been amply demonstrated that the properties of the contractile proteins are a function of their charge and ionic environment[6] and it is probable that changes in micellar geometry would likewise influence their function. An exact molecular model for the contractile unit in muscle is still a matter of controversy, although recent speculations place great emphasis upon the size, shape and flexibility of the myosin substructure.[20] It is tempting to speculate that the changes observed in the molecular configuration of myosin and actomyosin in cardiac failure are specifically related to the ability of the myofibril to convert the energy of cellular ATP into mechanical work.

The role of digitalis glycosides in influencing the contractility of the myocardium is pertinent to this discussion. It is generally agreed that digitalis is of little value in the treatment of uncomplicated high output failure in which the heart appears unable to maintain normal energy production. On the other hand, the digitalis glycosides are of great value in the treatment of low output failure in which the biochemical defect appears to reside in the contractile proteins. Digitalis increases cardiac work in low output failure without significant increases in coronary flow, oxygen consumption or substrate extraction.[21,22] In normal subjects digitalis may actually decrease stroke work and efficiency. Although some of the effects of digitalis upon oxidative reaction in cardiac muscle have been observed, these effects are most prominent in tissue slices and other *in vitro* preparations and probably bear little or no relationship to its activity in the intact animal in which inotropic effects can be demonstrated without changes in respiration.

[13] Ellenbogen, E. and Olson, R. E. Studies on the contractile proteins of the dog heart. *Federation Proc.*, 14: 207, 1955.

[14] Parrish, R. G. and Mommaerts, W. F. H. M. Studies on myosin. *J. Biol. Chem.*, 209: 901, 1954.

[15] Olson, R. E. Cardiac metabolism in congestive heart failure. Symposium on the pathological physiology of congestive failure. Boston University School of Medicine, Boston, Mass., October 10, 1955.

[16] Benson, E. S. Composition and state of protein in heart muscle of normal dogs and dogs with experimental myocardial failure. *Circulation Res.*, 3: 221, 1955.

[17] Mihalyi, E. and Szent-Gyorgyi, A. G. Trypsin digestion of muscle proteins. *J. Biol. Chem.*, 201: 189, 1953.

[18] Gergely, J., Gouvea, M. A. and Karibian, D. Fragmentation of myosin by chymotrypsin. *J. Biol. Chem.*, 212: 165, 1955.

[19] von Muralt, A. L. and Edsall, J. T. Studies on the physical chemistry of muscle globulin. *J. Biol. Chem.*, 89: 315, 1930.

[20] Morales, M. F., Botts, J., Blum, J. J. and Hill, T. L. Elementary processes in muscle action: an examination of current concepts. *Physiol. Rev.*, 35: 475, 1955.

[21] Bing, R. J., Maraist, J. F., Dammann, J. F., Draper, A., Jr., Heimbecker, R., Daley, R., Gerard, R. and Calazel, P. The effect of strophanthus on coronary blood flow and cardiac oxygen consumption of normal and failing hearts. *Circulation*, 2: 513, 1950.

[22] Olson, R. E., Roush, G. and Liang, M. M. L. Effect of acetyl strophanthidin upon the myocardial metabolism and cardiac work of normal dogs and dogs with congestive heart failure. *Circulation*, 12: 755, 1955.

One can only speculate at this time about the mechanism by which digitalis exerts its pharmacologic effect. *In vitro* effects of cardiac glycosides upon the spiralling of actomyosin,[23] the polymerization of actin[24] and the molecular size of myosin[13] have been observed. The order of magnitude of the dose required in man for digitalization (10 to 30 μg./kg.) is so small as to make a catalytic role at the enzyme level practically a certainty. Since digitalis decreases cardiac output and efficiency in the normal heart, it is possible that the cardiac aglycones, which are steroids, act as antimetabolites to certain physiologic sterols or steroids that determine the state of aggregation of myosin *in vivo* and thus influence contractility. Lipoprotein complexes of the heart and other tissues are probably formed through the utilization of secondary linkages such as hydrogen bonds. If in failure the normal lipoprotein structure of myosin is altered or dissociated, the addition of a cardiac glycoside might serve to restore a more nearly physiologic state.

The hydrogen bonding of myosin and of other proteins is extensive and could provide the basis for extensive changes in size and shape under the influence of both physiologic and pathologic stimuli and of endocrine and pharmacologic agents. The speculation that the cardiac glycosides influence protein structure through physical-chemical rather than purely chemical effects is consistent with the pharmacologic fact that a relatively large change in specific chemical configuration of sugar and steroid, notwithstanding of course certain basic requirements for biologic activity such as the C^{14}-hydroxyl and the C^{17}-lactone ring, can be tolerated in this series of compounds without loss of biologic activity. It would also explain other diverse manifestations of digitalis activity upon polarization and permeability of membranes[25] and upon surviving tissue slices.[26] Further, it might be surmised that if digitalis acts by influencing the physical-chemical state of selected cardiac proteins including myosin, it is not inconceivable that other steroids including the steroid hormones may act by influencing the physical-chemical state of other proteins in tissues under their control and perhaps thus regulate their biologic activity.

Further research is necessary to verify or disprove these hypotheses. The conception of heart failure as a manifestation of specific biochemical lesions in the myocardium should lead to investigations which eventually will define with precision the molecular pathogenesis of this disease.

ROBERT E. OLSON, M.D.
University of Pittsburgh
Graduate School of Public Health
Pittsburgh, Pennsylvania

[23] MALLOV, S. and ROBB, J. S. Behavior of actomyosin threads. *Federation Proc.*, 8: 104, 1949.

[24] HORVATH, I., KIRALY, C. and SZERB, J. Action of cardiac glycosides on the polymerization of actin. *Nature*, 164: 792, 1949.

[25] SZENT-GYORGYI, A. Chemical Physiology of Contraction in Body and Heart Muscle. New York, 1953. Academic Press.

[26] WOLLENBERGER, A. The energy metabolism of the failing heart and the metabolic action of the cardiac glycosides. *J. Pharmacol. & Exper. Therap.*, 97: 311, 1949.

Clinical Studies

Relationship of Immune Response to Group A Streptococci to the Course of Acute, Chronic and Recurrent Rheumatic Fever*

GENE H. STOLLERMAN, M.D., ARTHUR J. LEWIS, M.D., IRWIN SCHULTZ, M.D. *and* ANGELO TARANTA, M.D.

Irvington-on-Hudson, New York

IMPROVED immunochemical methods have made possible the demonstration of an antecedent group A streptococcal infection in virtually every new attack of acute rheumatic fever.[1,2] Once a rheumatic attack is initiated by the streptococcus, however, the course of the disease is unpredictable. It may end abruptly, enter a subacute or chronic phase, or relapse at a later date. Frequently it is not clear whether a given clinical cycle or exacerbation of the disease represents part of the course of a single attack or whether the disease is being reactivated or perpetuated by subclinical streptococcal infection. The present study attempts to clarify this problem by relating the immune response to streptococcal infection to the clinical course of acute and chronic rheumatic fever, and to relapses of the disease.

MATERIALS AND METHODS

Observations were made upon the clinical course of rheumatic fever in 580 patients hospitalized at Irvington House during the past four years under conditions in which the factor of streptococcal infection could be closely evaluated by routine throat cultures made at regular intervals, and by serial determinations of the serum titer of three streptococcal antibodies; antistreptolysin O, antistreptokinase and antihyaluronidase. During the first year of the study continuous chemoprophylaxis was not administered but the streptococcus was eradicated whenever its presence was detected by throat cultures made at weekly intervals. During the latter three years of the study continuous chemoprophylaxis was administered to all patients. Most patients received monthly intramuscular injections of 1.2 million units of benzathine penicillin.[3,4] Others received daily oral doses of either 1.0 gm. of sulfadiazine or 200,000 units of buffered penicillin G.

Beta hemolytic streptococci were isolated by direct inoculation of 5 per cent sheep blood agar. Attempts were made to group and type all strains of beta hemolytic streptococci by standard methods.[5] Determinations of the serum titer of antistreptolysin O were made by the method of Rantz and Randall, with minor modifications.[6] Streptococcal antihyaluronidase was assayed in sera by the turbidimetric method of Harris and Harris.[7] Antistreptokinase determinations were made by the quantitative method of Christensen.[8] Erythrocyte sedimentation rates (ESR) were measured by the method of Wintrobe, with corrections for variations of hematocrit according to standard tables. Tests for the presence of C-reactive protein (CRP) were carried out by the method of Anderson and McCarty.[9] Antibody determinations were made upon patient's sera which had been stored in a frozen state at −20°c. These sera were obtained from serial bleedings obtained weekly during the acute stage of the disease and at monthly intervals during convalescence. To determine changes in the level of a specific antibody in the same patient over a given period of time, stored samples of serum were thawed and the titers of antibody in all specimens were determined simultaneously on the same day. This was done to minimize variation due to technical errors. By this method a rise in titer of two dilution increments (two tubes) in any one of the tests employed was considered significant.

All of the patients included in this study met the criteria of Jones[10] for the diagnosis of rheumatic fever at some time during the course of their disease. Patients were grouped as follows (Table 1):

* From the Irvington House and the Department of Medicine, New York University College of Medicine, New York, New York. Supported in part by a grant from the Masonic Foundation for Medical Research and Human Welfare.

Group I was composed of patients considered to have a single "monocyclic" attack of rheumatic fever in which all clinical and laboratory evidence of rheumatic activity appeared to have subsided within six months of the onset of the disease. There were 461 patients in this group.

TABLE I

ANALYSIS OF THE COURSE OF RHEUMATIC FEVER

592 Admissions to Irvington House (580 Patients)

Group I	
Single "monocyclic" attack	461
Group II	
New attack after streptococcal infection:	
At Irvington House	5
Readmission to Irvington House	12
Group III	
Chronic rheumatic fever	25
Group IV	
Relapse without streptococcal infection:	
Frank clinical	34
Low-grade clinical	16
Group V	
Isolated manifestations:	
"New" after acute attack	
Chorea	22
Erythema marginatum	7
"Pure" without acute attack	
Chorea	22
Nodules	1

Group II consisted of those patients considered to have a new attack of rheumatic fever following a new streptococcal infection. There were five patients in whom such a new attack was observed while they were hospitalized at Irvington House and twelve who had been previously discharged from the hospital and were readmitted at a later date because of a new attack of rheumatic fever.

Group III were patients who were considered to have chronic rheumatic fever when a major clinical manifestation of the disease persisted for longer than six months. Twenty-five patients in this series were so classified.

Group IV were patients in whom a relapse of clinical manifestations of rheumatic fever occurred following withdrawal of antirheumatic therapy (aspirin, cortisone or ACTH) in the absence of evidence of new streptococcal infection. There were thirty-four patients in whom such relapses were considered to reflect "frank rheumatic activity" because of the reappearance of at least one major and two minor manifestations of rheumatic fever (criteria of Jones). There were sixteen patients in whom the relapse was considered of "low grade" severity because of the reappearance of at least two minor manifestations, such as fever, elevation of erythrocyte sedimentation rate, presence of C-reactive protein in the blood[11] or prolongation of P-R interval of the electrocardiogram.

Group V consisted of those patients who, following apparently complete subsidence of the acute rheumatic attack, subsequently developed either Sydenham's chorea or erythema marginatum as an isolated manifestation unassociated with any evidence of inflammation (fever, elevation of the ESR, or appearance of CRP in the blood). There were twenty-two such cases of chorea and seven cases of erythema marginatum. In addition there were twenty-one patients with "pure" Sydenham's chorea in whom there was no evidence of other rheumatic manifestations either prior to or concurrent with the episode of chorea. One patient in the series manifested typical rheumatic subcutaneous nodules (proved by biopsy) in the absence of prior or concurrent other rheumatic manifestations.

RESULTS

Relationship of Streptococcal Antibodies to the Acute Rheumatic Attack. There were eighty-eight patients from whom serum samples could be obtained within two months of the onset of clinical manifestations of acute rheumatic fever. (Fig. 1.) The initial determination of antistreptolysin O (ASO) exceeded a value of 200 units per cc. of serum in 78 per cent of this group. Nineteen of twenty of these patients who were observed within one month of the onset of rheumatic symptoms were found to have an ASO titer exceeding 200 units/cc. The remaining patient had a significant rise of at least two antibodies, but the initial levels were very low and the rise in antibody titer did not exceed the arbitrary value of 200 units/cc. which was set as an upper limit of "normal."

The titer of antihyaluronidase (AH) in the same serum samples was found to be elevated to 256 units/cc. or higher with the following frequency: within one month of the onset of rheumatic fever, 70 per cent; within two months, 68 per cent. An elevation of the titer of either antibody (ASO or AH) above 200 units/cc. was observed in 90 per cent of all patients studied within two months of the onset of the disease. (Fig. 1.)

When antistreptokinase determinations were included in the study of the same sera of this group, and a titer of more than 200 units per cc. was accepted as an upper limit, the percentage of patients whose serum showed an elevation of at least one of the three antibodies was increased to 95 per cent. This figure approached 100 per cent the earlier in the rheumatic attack the patient was studied. (Fig. 1.) The range of titers of the antibodies studied at various intervals

TABLE II

PERCENTAGE DISTRIBUTION OF ANTIBODY TITERS* AT MONTHS INDICATED FROM ONSET OF RHEUMATIC FEVER

Antibody Titer units/cc.	1 Month			2 Months			6 Months			12 Months		
	ASO	AH	ASK	ASO	AH	ASK	ASO	AH	ASK	ASO	AH	ASK
0–200	5	30	30	26.5	32.4	47.2	66.1	67.8	86.4	79.5	75	81.8
250–333	50	20	25	41.2	23.5	23.5	30.5	20.3	10.2	20.5	18.2	18.2
400–833	35	35	15	26.5	29.4	23.5	3.4	8.5	3.4	0	4.5	0
833–1280	10	15	30	5.8	14.7	5.8	0	3.4	0	0	2.3	0
>1280	0	0	0	0	0	0	0	0	0	0	0	0
No. patients studied	20	20	20	68	68	68	59	59	59	44	44	44

* ASO, antistreptolysin O titer; AH, antihyaluronidase; ASK, antistreptokinase.

from the onset of the acute rheumatic attack is shown in Table II.

The rate of fall of the titer of each antibody was followed during the course of the acute disease and during convalescence while antistreptococcal chemoprophylaxis was maintained. (Table II.) The antistreptolysin O titer was above 200 units/cc. in 95 per cent of twenty patients observed within the first month of the attack and in 74 per cent of sixty-eight patients observed during the second month. This percentage fell progressively to 34 six months after the onset of the disease and to 16 at the end of one year. Similar patterns were observed for the rate of fall of the other two antibodies studied. (Fig. 2.)

New streptococcal infection was considered to be excluded by a continuous fall in the titer of all three antibodies and by the absence of streptococci from the throats of patients receiving antibiotic prophylaxis. The subsequent course of the disease, with regard to severity, duration or cardiac involvement, bore no noticeable relationship to the rate of decline of antibody or to the level of antibody observed initially.

Chronic Rheumatic Fever. An attack of rheumatic fever in which clinical manifestations persisted longer than six months was classified as "chronic." The behavior of the three antibodies measured was studied in a group of twenty-three patients so classified.

Despite the persistence of rheumatic activity there was a progressive decline in the percentage of patients whose antibody titers were elevated at the time observations were begun. (Fig. 3.) The rate of decline was comparable to that demonstrated in patients with monocyclic attacks of rheumatic fever (Group I). There was, therefore, no evidence that chronic rheumatic fever was associated with a greater or more persistent elevation of the antibodies studied. In this series, clinically active rheumatic fever persisted for at least twenty-eight months in some patients without evidence of new, intercurrent streptococcal infection. Because many of these patients had been suffering from rheumatic fever for several months before admission to Irvington House, the initial antibody levels which could be determined were not as frequently elevated as in the case of those patients in whom observations were made closer to the onset of the disease. Indeed, it was usually possible to correlate roughly the levels of antibody initially encountered with the approximate duration of the active rheumatic process before the patient's admission to Irvington House. For example, those patients in whom serum antibody titers were found to be low upon admission to Irvington House were known to have had active rheumatic fever of at least several months' duration.

Relapses of Rheumatic Fever in Relation to New Streptococcal Infection. This study afforded the opportunity to determine, by all clinical and laboratory criteria employed, whether relapses of the disease following a period of apparent complete quiescence could be related to new streptococcal infection. Patients who had completed a course of antirheumatic therapy with either aspirin or cortisone (usually six to twelve weeks) and in whom all evidence of the active

disease appeared to be completely suppressed, were followed closely for evidence of relapse. A frank, clinical relapse was considered to have occurred when at least one major and two minor manifestations of rheumatic fever reappeared in the absence of any evidence of new streptococcal infection.

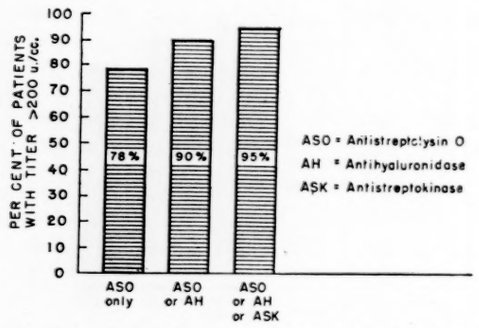


FIG. 1. Detection of recent streptococcal infection in eighty-eight patients studied within two months of onset of rheumatic fever.

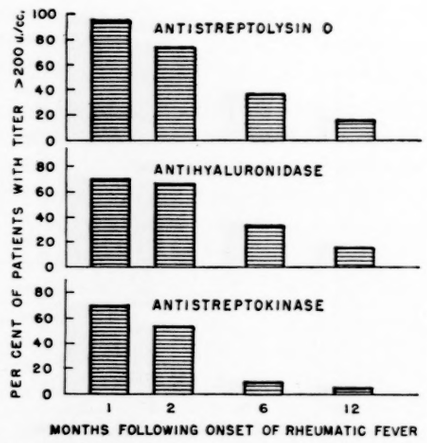


FIG. 2. Rate of fall of elevated antibody titers following an attack of rheumatic fever.

Of thirty-eight frank, clinical relapses observed in thirty-four patients twenty-two occurred within the first week following termination of treatment and eight within the second week. Six more developed within the next three weeks. Two relapses occurred between the seventh and eighth week (fifty and fifty-four days, respectively). Therefore, approximately 95 per cent of relapses following termination of therapy occurred within a month. In some instances the disease remained dormant for as long as eight weeks before exacerbating. (Fig. 4.)

An additional eighteen patients showed a relapse of low grade rheumatic activity following termination of antirheumatic therapy (reappearance of at least two minor manifestations of the disease, such as positive test for CRP and elevation of the ESR). Such relapses all appeared within twenty-five days of the termination of therapy.

TABLE III
RECURRENCE OF RHEUMATIC FEVER AFTER STREPTOCOCCAL INFECTIONS—GROUP II

	At Irvington House	Readmission to Irvington House
No. of patients	5	12
Positive throat cultures	5	.....
Clinical pharyngitis	4	.....
No. of patients with rise in titer:*		
Antistreptolysin O	2	11
Antistreptokinase	3	8
Antihyaluronidase	3	8
Any one of above	4	12
Interval between attacks	2 mo. to 3 yr.	2 mo. to 3 yr.

* Sera not available for study in one patient.

Beyond a period of eight weeks following withdrawal of aspirin or cortisone the reappearance of a new cycle of frank clinical rheumatic activity was always found to be associated with demonstrable evidence of new streptococcal infection. Seventeen patients in whom recurrence of rheumatic fever developed more than two months following the subsidence of rheumatic activity were studied for evidence of intercurrent streptococcal infection. Five of these patients had a recurrence of this nature while under observation at Irvington House and were among the group who did not receive continuous chemoprophylaxis during the first year of the study. In an additional twelve patients a new attack developed after they were discharged from Irvington House to the care of other agencies and were readmitted subsequently.

Of the five patients with recurrences observed at Irvington House all had been found to have a

throat culture positive for group A streptococci prior to the recurrence and four of the five had associated clinical signs of pharyngitis preceding the new rheumatic episode. Sera available for study in four of these patients showed a rise in at least one of the three antibodies studied. The

TABLE IV
PER CENT OF PATIENTS WITH ANTIBODY TITER >200 UNITS/CC. AT MONTHS INDICATED FROM ONSET OF RHEUMATIC FEVER

Antibody	1 Month	2 Months	6 Months	12 Months
Antistreptolysin O	95	73.5	33.9	20
Antihyaluronidase	70	67.6	32.2	25
Antistreptokinase	70	52.8	86	19
Any one of three antibodies	95	94.1	52.7	38.6
No. patients studied	20	68	59*	44*

* Represents same patients studied during the first or second months.

interval of apparent freedom from rheumatic activity between attacks was two months to three years. (Table III.)

Of the twelve patients admitted to Irvington House for the second time because of a recurrence of rheumatic fever all had evidence of a significant rise in the titer of at least one of the three antibodies studied. In this group the sera that had been obtained during the patient's previous admission had been stored in a frozen state and was available for determination of the level of each antibody at the termination of the original attack. (Table IV.)

An exception to these findings was the frequent late appearance of chorea as an isolated manifestation, without other major or minor manifestations of rheumatic activity. These patients are considered in detail in another report that analyzes the relationship of chorea to streptococcal infection.[12] Such instances of the delayed appearance of "isolated" chorea were observed in nineteen patients. Similarly, erythema marginatum appeared as a late, isolated manifestation in seven patients in whom no evidence of new streptococcal infection was demonstrated. In these instances there was no associated evidence of systemically active disease which might be inferred by such findings as fever, elevation of the ESR or a positive test for C-reactive protein in the blood. It is also noteworthy that in patients in whom frank rheumatic activity persisted over long periods of time exacerbations of the disease process occasionally developed without demonstrable new streptococcal infection.

COMMENTS

For purposes of analysis an upper limit of the usual range of serum antibody titer in normal

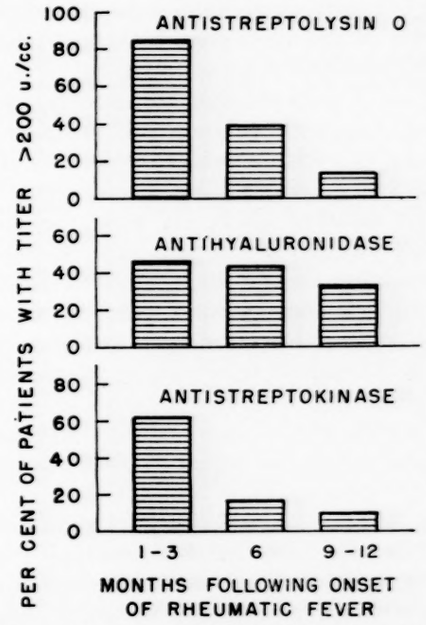


FIG. 3. Rate of fall of elevated antibody titers in chronic rheumatic fever in twenty-three patients.

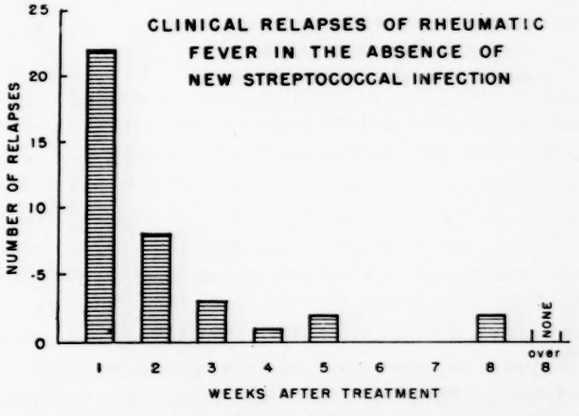


FIG. 4. Thirty-eight clinical relapses in thirty-four patients with acute rheumatic fever following withdrawal of treatment with aspirin or cortisone.

children was set at 200 units/cc. The age range and geographical location of the normal group selected from previous studies were similar to that of the children with rheumatic fever included in this study. Adequate data are available to justify selection of this upper limit for the

values of antistreptolysin O[13] and antihyaluronidase.[14] There have not been similarly extensive studies of the normal range of distribution of antistreptokinase titers, as measured by the quantitative method of Christensen employed in this study. Acceptance of an upper limit of 200 units/ml. for the serum titer of antistreptokinase is therefore tentative. From the parallel behavior of this antibody, however, it appears that this estimate is reasonable.

The data indicate that the antibodies measured in rheumatic subjects fall to levels below 200 units/ml. with considerable regularity after one year of freedom from streptococcal infection. In another study[4] it was demonstrated that approximately 97 per cent of rheumatic subjects have serum titers of ASO below 200 units/ml. after two to three years of continuous protection from infection with group A streptococci. It is reasonable to assume, therefore, that elevation of any of the three antibodies studied to levels significantly higher than 200 units/ml. represents relatively recent exposure to streptococcal infection.

This study adds additional evidence to support the contention that immunologic evidence of antecedent streptococcal infection may be found in every new attack of acute rheumatic fever. By the rate of fall of the antibodies studied it is apparent, however, that such measurements must be made early in the course of the disease to detect this relationship consistently. The low antibody titers which are frequently observed when low-grade rheumatic carditis is discovered at the initial clinical examination are usually a reflection of the long prior duration of the subclinical rheumatic process. This is often the case when the appearance of Sydenham's chorea or erythema marginatum first calls attention to the presence of the low-grade rheumatic process.[12]

Although elevated initially, the serum levels of the antibodies studied bear no relationship to the subsequent course of the rheumatic process. A uniform fall in titer was observed both in patients with mild rheumatic fever of brief duration and in those in whom the disease entered a chronic phase.

The factors relating to the development of chronic rheumatic fever remain obscure. This study leaves little doubt that once initiated by group A streptococcal infection rheumatic fever may persist unabated or undulate in intensity, exacerbating and remitting, for remarkably long periods without the additional insult of intercurrent streptococcal exposure. Fortunately, such attacks constitute a relatively small proportion of the total number observed. In the absence of new streptococcal infection the active rheumatic process appeared to be clinically arrested within six months of its onset in 95.7 per cent of the 580 patients studied. It may be of significance that in this series chronic rheumatic fever followed the initial attack of the disease only once. Of the remaining twenty-two patients all had suffered at least one previous rheumatic attack and frequently there had been several distinct prior rheumatic episodes.

Following suppressive treatment of rheumatic fever with aspirin or cortisone the disease appears to be capable of relapsing "spontaneously" (without new streptococcal infection) as late as eight weeks after the termination of therapy. Frank clinical relapses were observed in some patients with severe rheumatic heart disease after even longer latent periods. In such cases, however, laboratory evidence of relapse (reappearance of C-reactive protein in the blood and rise of ESR) or low-grade clinical signs (tachycardia during sleep, or fever) invariably reappeared within two months of the withdrawal of aspirin and cortisone.

Every relapse of frank rheumatic activity that appeared more than two months after apparent complete subsidence of rheumatic activity was associated with evidence of antecedent new streptococcal infection. During the period of study recurrences of rheumatic fever were not observed following a wide variety of acute infectious diseases, bone fractures, immunization procedures (with diphtheria and tetanus toxoid), intravenous injections of typhoid vaccine, etc.; only after a new group A streptococcal infection.

The occasional exceptional behavior of Sydenham's chorea, or erythema marginatum, deserves special comment and study. All patients in this series in whom chorea developed at any time during their rheumatic attack have been analyzed with respect to the factor of streptococcal infection and the results are presented in another report.[12] It appears likely that Sydenham's chorea sometimes occurs as a more delayed sequel to streptococcal infection than are the other manifestations of rheumatic fever.

Some practical clinical inferences may be drawn from the data presented. We have regarded the two months following termination of a course of treatment with aspirin or cortisone

(usually administered for six to twelve weeks) as the critical period required to observe the patient for signs or symptoms of a spontaneous relapse of the disease. After this interval, and providing that successful continuous chemoprophylaxis against new streptococcal infection is maintained, and adequate recovery of cardiac reserve has been gained, it has been found safe to return patients to normal community life with little fear of clinical relapse. The potentialities for the subsequent healing or progression of cardiac lesions in such patients has been discussed elsewhere.[15]

The rate of fall of the antibodies studied may serve as a guide to the efficiency of antistreptococcal prophylactic measures maintained in rheumatic subjects to prevent recurrences of the disease. Continuous prophylaxis with antibiotics by current methods,[16] and the use of the antibody measurements employed in this study, may make it possible to evaluate the natural history of rheumatic fever and rheumatic heart disease with reasonable assurance that the factor of intercurrent streptococcal infection has been excluded.

SUMMARY

The clinical course of rheumatic fever in relation to the factor of streptococcal infection was studied in 580 patients.

A high initial titer of either antistreptolysin O, antistreptokinase or antihyaluronidase was found in the sera of 95 per cent of patients who could be studied within the first two months of onset of the rheumatic attack. This figure approached 100 per cent in those studies made closer to the onset of the attack. The rate of fall of these three antibodies bore no relationship to the subsequent clinical course of the rheumatic attack.

Following suppressive therapy with aspirin or cortisone, all frank relapses of the disease which were unassociated with new streptococcal infection occurred within two months. Thereafter, the reappearance of frank rheumatic fever was invariably associated with immunologic evidence of new streptococcal infection.

The implications of these findings for the management of the rheumatic subject and the effect of antistreptococcal prophylaxis upon the natural history of the disease are discussed.

REFERENCES

1. McCarty, M. The immune response in rheumatic fever. In: Rheumatic Fever, edited by Lewis Thomas. Minneapolis, 1952. University of Minnesota Press.
2. Stetson, C. A., Jr. The relation of antibody response to rheumatic fever. In: Streptococcal Infections, edited by Maclyn McCarty. New York, 1954. Columbia University Press.
3. Stollerman, G. H. and Rusoff, J. H. Prophylaxis against group A streptococcal infections in rheumatic fever patients with new repository penicillin. *J. A. M. A.*, 150: 1571, 1952.
4. Stollerman, G. H., Rusoff, J. H. and Hirschfeld, I. Prophylaxis against group A streptococci in rheumatic fever patients by the use of single monthly injections of benzathine penicillin G. *New England J. Med.*, in press.
5. Swift, H. F., Wilson, A. T. and Lancefield, R. C. Typing group A streptococci by M precipitation reaction in capillary pipettes. *J. Exper. Med.*, 78: 127, 1943.
6. Rantz, L. A. and Randall, E. Modification of technic for determination of antistreptolysin titer. *Proc. Soc. Exper. Biol. & Med.*, 59: 22, 1945.
7. Harris, S. and Harris, T. N. The measurement of neutralizing antibodies to streptococcal hyaluronidase by a turbidimetric method. *J. Immunol.*, 63: 233, 1949.
8. Christensen, J. Methods for measuring the activity of components of the streptococcal fibrinolytic system and streptococcal desoxyribonuclease. *J. Clin. Investigation*, 28: 163, 1949.
9. Anderson, H. C. and McCarty, M. Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever. *Am. J. Med.*, 8: 445, 1950.
10. Jones, T. D. The diagnosis of rheumatic fever. *J. A. M. A.*, 126: 481, 1944.
11. Stollerman, G. H., Glick, S., Patel, D. J., Hirschfeld, I. and Rusoff, J. H. Determination of C-reactive protein in serum as a guide to the treatment and management of rheumatic fever. *Am. J. Med.*, 15: 645, 1953.
12. Taranta, A. and Stollerman, G. H. The relationship of Sydenham's chorea to infection with group A streptococci. *Am. J. Med.*, 20: 170, 1956.
13. Rantz, L., Randall, E. and Rantz, H. H. Antistreptolysin "O." A study of this antibody in health and in hemolytic streptococcus respiratory disease in man. *Am. J. Med.*, 5: 3, 1948.
14. Harris, S. and Harris, T. N. Serologic response to streptococcal hemolysin and hyaluronidase in streptococcal and rheumatic infection. *J. Clin. Investigation*, 29: 351, 1950.
15. Stollerman, G. H. Potentialities for and limitations in the control of rheumatic fever by prophylactic measures. *J. Chronic Dis.*, 1: 216, 1955.
16. Stollerman, G. H. Use of antibiotics in the prevention of rheumatic fever. *Am. J. Med.*, 17: 757, 1954.

The Relationship of Sydenham's Chorea to Infection with Group A Streptococci*

ANGELO TARANTA, M.D. *and* GENE H. STOLLERMAN, M.D.
Irvington-on-Hudson, New York

THE relationship between rheumatic fever and Sydenham's chorea (chorea minor) has been a controversial subject almost since the time of the first adequate clinical description of these disorders. As early as 1820 the observation was made that "chorea minor sometimes alternates with acute rheumatism."[1] In the following years extensive statistical studies[2] established the fact that chorea, polyarthritis and carditis tend to occur in the same patients with a frequency far too high to be coincidental. Consequently, chorea minor and rheumatic fever, which originally had been described independently, came to be considered as distinct manifestations of the same syndrome of unknown etiology.

Several aspects of the relationship of chorea to rheumatic fever remain puzzling.[3–7] Chorea frequently appears as an isolated finding, unaccompanied and unpreceded by other rheumatic manifestations. Often, in such instances, no evidence of rheumatic inflammation is found. The erythrocyte sedimentation rate is normal and the presence of C-reactive protein in the blood cannot be detected.[8] Furthermore, it has commonly been observed that immunologic evidence of recent streptococcal infection (antistreptolysin O titer) may be absent at the onset of chorea.[9]

The role of group A streptococcal infection in initiating rheumatic fever has recently been established and immunologic evidence of antecedent streptococcal infection has been demonstrated at the onset of virtually every new rheumatic attack.[9] The absence of such evidence in some cases of chorea minor suggests either that these cases are unrelated to group A streptococcal infection or that chorea minor may appear as a late manifestation of the rheumatic attack at a time when most streptococcal antibodies usually measured in serum have fallen to low values.

The present study attempts to test the latter hypothesis by analyzing the case histories of ninety-two patients with chorea who were observed under conditions in which the factor of streptococcal infection could be closely controlled and specific streptococcal serum antibodies studied.

MATERIALS AND METHODS

The clinical material and laboratory methods employed are described in another study.[9] The medical histories of ninety-two patients with Sydenham's chorea, who were hospitalized in recent years at Irvington House, were reviewed. The immunologic studies on the late appearance of chorea were made in patients having their first attack of chorea. It was difficult to evaluate patients who had a previous attack of chorea. Such patients persistently have low grade choreiform activity, with remissions and exacerbations. Therefore, the distinction between a new attack of chorea and an exacerbation of chronic chorea often is not clear-cut. The patients were grouped for study as follows:

Group I (twenty-two patients) had chorea as the only clinical rheumatic manifestation. This group was designated as "pure" chorea. Because of the limited number of patients of this type available in our own series an additional source of similar material was sought in the files kept for the past ten years by the Department of Pediatrics of Bellevue Hospital.† Forty-one patients with pure chorea in whom determinations of antistreptolysin O (ASO) titers had been made were available for study.

Group II (seventy patients) consisted of patients with chorea who had had some additional major manifestation of rheumatic fever at some time and therefore were definitely rheumatic subjects. In this

† We are indebted to Dr. Janet S. Baldwin for these data.

* From the Irvington House, Irvington-on-Hudson, New York, and the Department of Medicine, New York University College of Medicine. Supported in part by a grant from The Masonic Foundation for Medical Research and Human Welfare, and by a grant-in-aid from the National Heart Institute, National Institutes of Health, U. S. Public Health Service.

group an analysis was made of the time of onset of chorea in relation to other rheumatic manifestations following the initial streptococcal infection.

To determine whether or not recent antecedent streptococcal infection occurred in all patients with chorea, studies of specific streptococcal antibodies were made as described in another study.[9] In addition, an analysis of streptococcal antibodies was made in the cerebrospinal fluid of as many patients with active chorea as possible. This was done to determine whether or not the titer of these antibodies might be disproportionately high in the central nervous system during active chorea.

RESULTS

Time Relationship of Onset of Chorea Minor to Onset of Polyarthritis, Polyarthralgia and Carditis in Same Patients. Of the histories of seventy patients who had chorea and some other manifestations of rheumatic fever, fifty-five were found to be sufficiently well documented to indicate clearly the time relationships between the onset of the various manifestions. (Fig. 1.)

It appeared that in most cases clear-cut rheumatic manifestations preceded chorea. In some instances chorea was the presenting symptom and other evidence indicative of rheumatic fever was noted at the time of examination. For example, the frequent apparent coincidence of the onset of carditis with the onset of chorea was striking. Closer analysis revealed, however, that in most cases the carditis was subclinical, no symptoms being described. The diagnosis of carditis was made when medical attention was first directed to the patient because of the appearance of choreiform activity. The actual duration of cardiac involvement cannot be clearly ascertained. To consider the onset of carditis as simultaneous with that of chorea might be misleading.

It is well known that chorea and typical migratory polyarthritis rarely occur together. Only one such case was noted in this series. Concurrent polyarthralgia and chorea on the other hand were commonly observed. In three patients with hemichorea the joint pains appeared only on the side of the body affected by chorea. These observations suggest that the polyarthralgia associated with chorea might differ from frank polyarthritis and might not represent merely a milder form of the latter.*

* Observations similar to ours were made by Mitchell[2] more than sixty years ago (he spoke of these cases as "painful chorea"). Little attention has been paid to this observation.

These studies indicate that when chorea and other rheumatic manifestations occur in the same patient over a relatively brief time interval, chorea tends to follow the other rheumatic manifestations. In some cases the interval between the onset of chorea and the onset of the rheumatic manifestation which preceded it was so long as to suggest that there might have been an intercurrent streptococcal infection which was responsible for the late occurrence of chorea. To test this possibility the following study was made.

Studies of Serum Antibodies in Patients in Whom Chorea Developed after Other Rheumatic Manifestations. The patients selected for this study were admitted to Irvington House during or shortly after a typical attack of rheumatic fever. They had no previous history of chorea. After two or more months of observation typical choreiform activity developed. During their stay at Irvington House monthly injections of 1.2 millions of benzathine penicillin were administered. Throat cultures obtained at regular intervals were negative for group A streptococci. Antistreptolysin O titer (ASO), antihyaluronidase (AH) and antistreptokinase (ASK) were determined monthly in samples of serum up to two months after the onset of chorea. These samples showed a consistent gradual fall of titers. In these patients there was therefore a period, after the onset of the rheumatic manifestation which preceded chorea and before the onset of chorea itself, in which the occurrence of a streptococcal infection could be ruled out with reasonable assurance. The duration of this "streptococcus-free" period preceding the onset of chorea was as follows: two months in four cases, three months in three cases, four months in one, five months in one, and seven months in one. The interval between the onset of the first rheumatic manifestation and the onset of chorea was actually greater, since the onset of rheumatic fever in most cases occurred a variable length of time prior to admission to Irvington House. The longest interval was nine months. No evidence is available, however, that streptococcal infections did or did not occur between the onset of rheumatic fever and the beginning of strict prophylaxis after admission to Irvington House.

It is of interest that of the ten patients studied (Fig. 2) all had significant elevation of at least one of the three streptococcal antibodies during or shortly after the initial manifestation of rheumatic fever. By the time chorea appeared, however, these titers had fallen, so that only four

of the ten patients still retained a high titer of antibodies. A typical case with long interval between the onset of the other rheumatic manifestations and the onset of chorea is shown diagrammatically in Figure 3.

By the time chorea developed many of these patients had completely recovered, by all clinical and laboratory criteria, from the previous rheumatic manifestation, so that the disease followed a typical "biphasic" pattern.

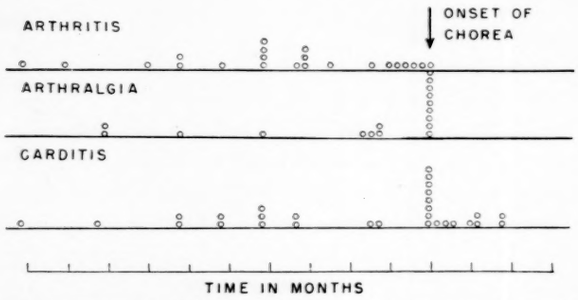


FIG. 1. Time relationship of the onset of chorea minor to the onset of polyarthritis, polyarthralgia and carditis in the same patients.

	ASO	AH	ASK
W.S.	333 *100*	256 *128*	640 *80*
M.R.	800 *100*	384 *128*	320 *160*
G.C.	625 *333*	768 *128*	640 *160*
E.McL.	500 *333*	192 *192*	320 *320*
R.A.	250 *125*	384 *196*	160 *80*
V.G.	333 *333*	384 *256*	40 *40*
Y.W.	250 *166*	48 *64*	80 *20*
M.U.	625 *625*	394 *96*	20 *20*
J.D.	333 *100*	48 *32*	80 *20*
C.M.	125 *125*	32 *32*	320 *80*

FIG. 2. Antistreptococcal antibodies in the serum of patients with chorea minor preceded by some other manifestation of rheumatic fever. The italic figure is the titer at the onset of chorea; the roman figure is the titer during or shortly after the other rheumatic manifestations which preceded chorea. ASO, antistreptolysin O; AH, antihyaluronidase; ASK, antistreptokinase. Titers expressed in units/cc.

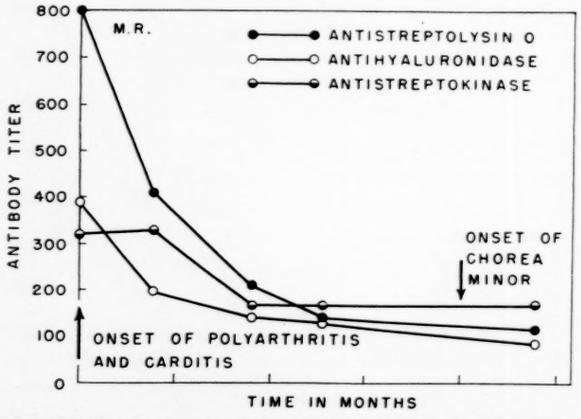


FIG. 3. Chorea minor appearing four months after the onset of polyarthritis and carditis without evidence of intercurrent streptococcal infection.

These studies show that chorea may occur as late as seven months after the onset of some other rheumatic manifestation, without intercurrent streptococcal infection. They also show that in patients who have had typical attacks of rheumatic fever associated with immunologic evidence of recent streptococcal infection chorea may subsequently develop at a time when the various streptococcal antibodies may have returned to normal levels.

Studies of Streptococcal Serum Antibodies in Patients with Pure Chorea. In patients who had pure chorea an attempt was made to determine, on the basis of the titer of streptococcal serum antibodies, whether or not recent streptococcal infection had occurred. The sera of ten of the twenty-two patients with pure chorea observed at Irvington House were available for study. All ten of these patients were studied within five months from the onset of choreiform activity and the onset of the disease in these instances appeared to be clear-cut. A titer of more than 200 ml. of antistreptolysin O (ASO) was found in eight of ten patients. (Table I.) Of the two remaining patients, one had an antihyaluronidase titer of 300 units per ml., the other a low titer of all three antibodies.

ASO determinations were made within five months of the onset of choreiform activity in a similar group of forty-one patients with pure chorea who had been observed on the Children's Cardiac Service, Bellevue Hospital. Twenty-seven of these patients (65.8 per cent) had an ASO titer greater than 200 units per cc. (Table II.) In reviewing these records it was noted that in thirty patients the ASO determinations had been made within the first month of choreiform activity. Of these thirty children, twenty-two (73.3 per cent) had titers of more than 200 units per cc. Of eleven patients studied later during the course of their attack of chorea, an elevation of ASO above the upper limit of normal was observed in only five (45.5 per cent).

These data indicate that the earlier after onset that the ASO titer determination is made the higher it tends to be in patients with pure chorea. However, the titers are somewhat lower than those observed in patients who are studied within the first month of onset of other rheumatic manifestations; in such patients the incidence of those showing ASO titers greater

than 200 units per cc. may exceed 95 per cent.[9] The incidence of elevated ASO titers in patients with pure chorea studied within one month of onset is very similar (73.3 per cent) to that observed in patients with rheumatic fever studied during the second month, namely, 73.5 per cent.[9]

TABLE I
ANTISTREPTOCOCCAL ANTIBODIES* IN THE SERUM OF PATIENTS WITH CHOREA MINOR AS THE ONLY CLINICAL MANIFESTATION (PURE CHOREA)

Patient	ASO	AH	ASK
P. O'C.	250	128	160
S. B.	250	512	320
P. J. B.	333	256	80
M. R.	166	64	160
P. N.	166	300	10
C. G.	333	768	640
M. McA.	333	1024	320
J. C.	250	512	640
L. M.	500	256	160
E. D.	500	196	640

* ASO, antistreptolysin 0; AH, antihyaluronidase; ASK, antistreptokinase. Titers expressed in units/cc.

TABLE II
ASO TITER IN PATIENTS WITH PURE CHOREA MINOR*

30 patients observed within one month from onset:	
ASO titer more than 200 units/cc	22 (73.3%)
ASO titer equal to or less than 200 units/cc	8 (26.7%)
11 patients observed between one and five months after onset:	
ASO titer more than 200 units/cc	5 (45.4%)
ASO titer equal to or less than 200 units/cc	6 (54.6%)

* Observations made at Bellevue Hospital, New York, N. Y.

Immunologic Studies in Cerebrospinal Fluid (CSF). Streptococcal antibodies in the cerebrospinal fluid of patients with chorea were investigated. ASO, AH and ASK were determined simultaneously in samples of CSF and serum of fifteen patients with active chorea and of fifteen patients with rheumatic fever without chorea. Undiluted CSF and low dilutions were always used. In this way antibodies were found in the CSF of four of the fifteen patients with chorea and in five of the fifteen patients without chorea. The CSF antibodies never exceeded a titer of two units per ml. In each case the antibodies present in the CSF were those which occurred in the highest concentration in the corresponding serum. No antibodies were found in the CSF unless the titer in the corresponding serum was at least 250 units per ml.

A comparison of antibodies in the cerebrospinal fluid of the chorea and the control group failed to show any significant difference. (Table III.)

TABLE III
COMPARISON OF STREPTOCOCCAL ANTIBODY IN THE SERUM AND SPINAL FLUID OF RHEUMATIC FEVER PATIENTS WITH AND WITHOUT CHOREA

	ASO (units/cc.)		AH (units/cc.)		ASK (units/cc.)	
	250 or >	<250	250 or >	<250	250 or >	<250
Serum titers:						
With chorea	7	8	7	8	3	12
Without chorea	8	7	7	8	4	10
	>1	<1	>1	<1	>1	<1
Cerebrospinal fluid titers:						
With chorea	2	13	2	13	1	13
Without chorea	1	14	3	12	2	12

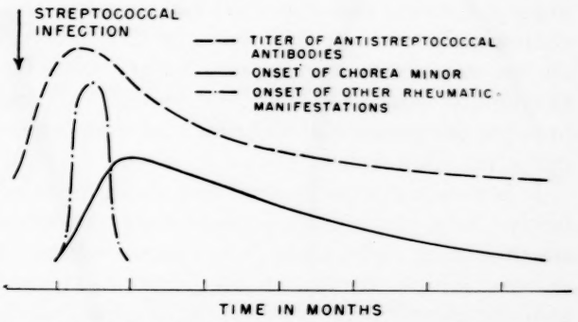


FIG. 4. Relationship among chorea minor, streptococcal infection and other rheumatic manifestations.

COMMENTS

A large statistical study of the seasonal incidence of rheumatic fever and of chorea minor[10] shows that the peak of maximal incidence of rheumatic fever falls in April and that of chorea in June. The month of minimal incidence of rheumatic fever is August and of chorea, November. Two other similar studies, made in different periods and different places,[11,12] show the same phenomenon, the peak of incidence of chorea following the peak of incidence of rheumatic fever by an interval of one to three months. The data we have collected suggest an interpre-

tation of these statistical findings, as well as of the relationship between streptococcal infections and chorea minor, and between chorea minor and other manifestations of rheumatic fever. A scheme representing this interpretation is presented in Figure 4.

When chorea occurs in patients who show other manifestations of acute rheumatic fever, its onset tends to follow the appearance of the other rheumatic manifestations. (Fig. 1.) The serologic studies show that a lag period of as long as seven months may intervene between the onset of rheumatic fever and the subsequent onset of chorea, without any new streptococcal infection. A causal relationship between a preceding streptococcal infection and the occurrence of chorea, even in these cases, is suggested by the fact that no new attack of chorea has been observed after more than nine months of a "streptococcus-free" regimen either in the course of this study or in cases encountered in a large rheumatic fever prophylaxis clinic.[13]

At the time chorea appears the streptococcal antibodies may have fallen to a normal level, although they were high during the preceding rheumatic manifestation. (Fig. 2.) These studies suggest that the latent period between the preceding streptococcal infection and the onset of chorea is usually considerably longer than the commonly observed latent or "silent" period between streptococcal infection and other rheumatic manifestations.

It is reasonable to assume that the same relatively long lag period intervenes between streptococcal infections and those cases of chorea which occur as an isolated clinical manifestation (pure chorea). It is conceivable that in at least some of these cases subclinical manifestations of rheumatic fever may have preceded the onset of chorea without being recognized and diagnosed.

The difference in the average lag period between streptococcal infection and chorea, and between streptococcal infections and other rheumatic manifestations, is in keeping with epidemiologic observations that the peak incidence of chorea follows that of acute rheumatic fever. Therefore, it is not surprising that polyarthritis and chorea seldom occur at the same time in the same patient.[10]

It seems pertinent to emphasize that the immunologic studies on the late appearance of chorea were made in patients in the course of their first attack of chorea. Patients who have had a previous attack of chorea frequently show persistent low grade choreiform activity, with remission and exacerbations. We have observed such exacerbations in patients receiving prophylaxis after a streptococcus-free period of as long as eleven months. The distinction between a new attack of chorea and exacerbation of chronic chorea often is not clear-cut. The situation is comparable to the cases of chronic carditis described elsewhere[9] in which the disease persists and fluctuates independently of new streptococcal infections.

The investigations on the antibody content of the cerebrospinal fluid failed to throw any light on the pathogenesis of the disease. The low titers found in the cerebrospinal fluid are probably the result of filtration of antibody from the blood. No difference was found between the chorea group and the control group, and the ratio of antibody in the CSF to that in the blood was comparable to that of CSF proteins to serum proteins.

Although analysis of a sufficient number of patients was not carried out, it is the authors' opinion that erythema marginatum behaves in very similar fashion to chorea with regard to its relatively late appearance in the course of the disease and occasional onset as an isolated manifestation long after other signs of rheumatic inflammation have subsided and streptococcal antibodies have returned to lower levels.

SUMMARY

The relationship between chorea minor and streptococcal infections was investigated by means of determination of streptococcal antibodies in patients with chorea. Fifty-one patients with chorea as the only clinical manifestation (pure chorea) were studied. The incidence of elevated streptococcal antibody titers, indicative of recent streptococcal infections, was higher in a group of thirty patients studied within one month from the onset of chorea (73.3 per cent) than in patients studied later (45.4 per cent). This incidence was, however, lower than in a comparable group of patients with other manifestations of rheumatic fever (95 per cent).

An analysis of the histories of fifty-five patients with chorea and other manifestations of rheumatic fever showed that the onset of chorea usually follows the onset of the other rheumatic manifestations. A longer lag period between streptococcal infections and chorea minor than between streptococcal infections and the other

most common manifestations of rheumatic fever was suggested by these studies.

Serial determinations of three streptococcal antibodies were made in ten patients in whom chorea started more than two months after the onset of another rheumatic manifestation. The possibility of intercurrent streptococcal infection was thereby ruled out. By the time chorea appeared, the streptococcal antibody titers were often low, although they were high initially.

The interpretation is advanced that the relatively long lag period between streptococcal infections and chorea may explain the lack of immunologic evidence of recent streptococcal infections in some cases of pure chorea. Some clinical and epidemiologic data are discussed in connection with this interpretation.

Immunologic studies of the cerebrospinal fluid in fifteen patients with chorea failed to yield information on the pathogenesis of the disease.

REFERENCES

1. Syllabus, or Outlines of Lectures on the Practice of Medicine. London, 1820. Cited by Osler.
2. Reviewed by Osler, W. On Chorea and Choreiform Affections. Philadelphia, 1894. P. Blakiston Son & Co.
3. Jones, T. D. and Bland, E. E. Clinical significance of chorea as a manifestation of rheumatic fever. J. A. M. A., 105: 571, 1935.
4. Gerstley, J. R., Wile, S. A., Falstein, E. I. and Goyle, M. Chorea: is it a manifestation of rheumatic fever? *J. Pediat.*, 6: 42, 1935.
5. Coburn, A. F. and Moore, L. V. The independence of chorea and rheumatic activity. *Am. J. M. Sc.*, 193: 1, 1937.
6. Usher, S. J. Chorea—relation to rheumatic fever and heart disease. *Canad. M. A. J.*, 39: 565, 1938.
7. Kagan, B. M. and Mirman, B. Sydenham's chorea, a syndrome for differential diagnosis. *J. Pediat.*, 31: 322, 1947.
8. Stollerman, G. H., Slick, S. J., Patel, D. J., Hirschfeld, I. and Rusoff, J. H. Determination of C-reactive protein in serum as a guide to the treatment and management of rheumatic fever. *Am. J. Med.*, 15: 645, 1953.
9. Stollerman, G. H., Lewis, A. J., Schultz, I. and Taranta, A. Relationship of the immune to group A streptococci to the course of acute, chronic and recurrent rheumatic fever. *Am. J. Med.*, 20: 163, 1956.
10. Gibson, S. In: Brenneman's Practice of Pediatrics. Hagerstown, Md., 1948. W. F. Prior Co.
11. Norgren, R. Sur le mode d'apparition de la chorée. *Acta paediat.*, 2: 159, 1923.
12. Hedley, O. F. Rheumatic heart disease in Philadelphia Hospitals. IV. Influence of season and certain meteorological conditions. *Pub. Health Rep.*, 55: 1809, 1940.
13. Stollerman, G. H. and Taranta, A. Unpublished observations.

Epidemiologic Studies on Antibiotic-Resistant Strains of Micrococcus Pyogenes*

ROBERT I. WISE, M.D., *Philadelphia, Pennsylvania*
CAROLINE CRANNY *and* WESLEY W. SPINK, M.D.
Oklahoma City, Oklahoma *Minneapolis, Minnesota*

DURING the past few years medical centers throughout the world have reported on the increasing incidence of antibiotic resistant strains of Micrococcus pyogenes. A similar experience has been encountered at the University of Minnesota Hospitals which has prompted the present report on a comparative study of coagulase positive strains of staphylococci isolated from different environmental groups. Particular interest was focused on antibiotic resistance and the bacteriophage types exhibited by the collected cultures.

THE CHANGING INCIDENCE OF STRAINS OF STAPHYLOCOCCI SUSCEPTIBLE TO ANTIBIOTIC ACTIVITY

It is difficult to compare reported studies on microbial resistance to antibiotics because of a number of factors, one of these being a lack of standardization of technics used in sensitivity tests. Furthermore, resistance is relative, and the interpretation of *in vitro* results for clinical purposes has undergone changes since the beginning of the antibiotic era from 1941 to 1942. This is due in part to the readily available supply of antibiotics and to the use of much larger doses of the drugs in treatment. The changing interpretation placed upon *in vitro* results can be ascertained from experience in our laboratory. In 1944, Spink et al.[1] reported on the results of the *in vitro* action of penicillin against strains of staphylococci isolated from human sources prior to the introduction of penicillin, stating that "8 of the strains or approximately 12 per cent were found to be quite resistant, requiring 0.4 units to 0.8 units of penicillin before growth was completely inhibited." In the light of subsequent experience these values fall well within concentrations that can be readily achieved in the body fluids of patients being treated with penicillin. Recognizing that all strains of bacteria possess some measurable degree of resistance to antibiotics, the sensitivity of strains of staphylococci in our laboratory has been classified for practical purposes in the following manner: (1) *sensitive*, inhibited by 1.0 unit or microgram of antibiotic per milliliter; (2) *moderately resistant*, inhibited by 1.0 to 10.0 units or micrograms per milliliter, and (3) *resistant*, inhibited by more than 10.0 units or micrograms per milliliter. Essentially the same tube dilution technic has been employed since 1941 in our laboratory for testing the *in vitro* sensitivity of staphylococci.

Many reports have emphasized the increasing frequency with which penicillin resistant strains of staphylococci were being encountered in infections of hospitalized patients. These studies, which have been reported by different investigators from medical centers in many parts of the world, are summarized in Table 1*a*. Initial observations on the susceptibility of staphylococci isolated prior to the advent of penicillin revealed that there were no strains resistant to 1.0 unit of penicillin per ml.[1,2] But as early as 1942 Rammelkamp and Maxon[3] reported increased resistance to penicillin with four strains of staphylococci isolated from patients during the course of penicillin therapy for localized infections. The increasing incidence of penicillin resistant strains was reported by others, and by 1945 the percentage of strains from hospitalized patients which were resistant to one unit of penicillin ranged from 13 per cent to 57 per cent.[4–7] In one hospital the percentage of resistant strains was reported to be 14.1 per cent in 1946, 38 per cent in 1947 and 59 per cent in 1948.[10] From 1951 to 1953 the percentage ranged from 64.7 per cent to 80.0 per cent.[8,20–24]

In contrast to the rising incidence of penicillin resistant strains of staphylococci, which have been isolated from hospitalized patients, there

* From the Department of Medicine, University of Minnesota Hospitals and Medical School, Minneapolis. Aided by a grant from Chas. Pfizer & Co., Inc., Brooklyn, New York.

has been no associated rise in the incidence of resistant strains obtained from outpatients and persons unassociated with hospitals. The reported results of studies in the latter group are listed in Table 1*b*. The incidence of penicillin resistant strains obtained from outpatients has varied from 0 to 36 per cent.

Studies of cultures of M. pyogenes isolated from the upper respiratory tracts of apparently healthy personnel in hospitals are listed in Table 1*c*. From 1949 to 1953 the incidence of resistant strains from this group varied from 30 per cent to 85 per cent. These results are similar to those obtained from studies of strains isolated from infected hospitalized patients, and indicate a possible epidemiologic relationship between the strains from the two groups. It can be concluded from these reports that a high incidence of penicillin resistant strains of staphylococci now exists in the nares and pharynges of members of hospital staffs and in infections of hospitalized patients. A lower incidence of similar strains has been noted which inhabits the nasopharynges of persons in outpatient groups.

TABLE I
INCIDENCE OF PENICILLIN RESISTANT STRAINS OF MICROCOCCUS PYOGENES

Year	Authors	Country	Per cent Resistant
	a. Hospitalized Patients		
Prior to 1942	Spink et al.[1]	U. S. A.	0
Prior to 1942	North and Christie[2]	Australia	0
1944	Rantz and Kirby[4]	U. S. A.	21.0
1945	Bondi and Dietz[5]	U. S. A.	13.9
1945	Gallardo[6]	U. S. A., India	12.9
1945	Plough[7]	U. S. A.	36.6
1946	Böe and Vogelsang[8]	Norway	15.9–54.5
1946	Blair et al.[9]	U. S. A.	8.8
1946	Barber and Whitehead[10]	England	14.1
1947	Barber and Whitehead[10]	England	38.0
1948	Barber and Whitehead[10]	England	59.0
1949	Summers[11]	England	50.0
1949	Beigelman and Rantz[12]	U. S. A,.	56.0
1949	Forbes[13]	England	68.4
1949	Martyn[14]	England	55.5
1949	Rountree and Thomson[15]	Australia	53.0
1949	Berger[16]	Austria	19.0–40.0
1949	Nichols and Needham[17]	U. S. A.	68.0
1950	Spink[18]	U. S. A.	55.0
1950	Cairnes and Summers[19]	England	78.0
1951	Böe and Vogelsang[8]	Norway	68.0
1951	Rountree[20]	Australia	80.0
1952	Finland and Haight[21]	U. S. A.	75.0
1952	Rountree and Thomson[22]	Australia	64.7
1953	Dowling et al.[23]	U. S. A.	69.0
1953	Miyahara et al.[24]	U. S. A.	76.0
	b. Outpatients		
1948	Barber and Whitehead[10]	England	29.4
1949	Barber et al.[25]	England	0.0
1949	Summers[11]	England	24.0
1949	Forbes[13]	England	12.5
1949	Rountree and Thomson[15]	Australia	4.0
1951	Thompson and Schwabacher[26]	England	20.0
1951	Vogelsang[27]	Norway	3.9
1952	Birnstingle et al.[28]	England	16.0
1952	Linsell[29]	England	18.6
1952	Rountree and Thomson[22]	Australia	36.0
1953	Dowling et al.[23]	U. S. A.	25.0
	c. Hospital Staff		
1949	Barber et al.[25]	England	83.0
1949	Rountree and Thomson[15]	Australia	32.0
1949	Rice and Lonargan[30]	U. S. A.	30.0
1951	Rountree and Barbour[31]	Australia	32.1
1953	Dowling et al.[23]	U. S. A.	85.0

TABLE II
INCIDENCE OF STREPTOMYCIN RESISTANT STRAINS OF MICROCOCCUS PYOGENES

Year	Authors	Country	Per cent Resistant
	a. Hospitalized Patients		
1948	Barber et al.[32]	England	0.0
1949	Forbes[13]	England	0.0
1949	Martyn[14]	England	3.7
1949	Rountree and Thomson[15]	Australia	16.5
1950	Spink[18]	U. S. A.	98.0
1951	Rountree[20]	Australia	45.0
1953	Miyahara et al.[24]	U. S. A.	37.0
	b. Outpatients		
1952	Rountree and Thomson[22]	Australia	10.8
	c. Hospital Staff		
1949	Rice and Lonargan[30]	U. S. A.	0.0

Observations on the incidence of strains of staphylococci resistant to streptomycin are presented in Table II. As in the case of penicillin, no resistant strains were encountered when this antibiotic was first introduced.[13,32] However, the incidence of resistant strains increased rapidly during the years from 1949 to 1953. By 1950 Spink[18] found 98 per cent of the strains studied at the University of Minnesota Hospitals to be resistant to 1 μg. of streptomycin per milliliter. Rountree and Thomson[22] in studying material from outpatients found only 10.8 per cent of strains to be resistant to streptomycin in 1952.

Reported results of studies with chlortetracycline (aureomycin) are shown in Table III. In 1949 no strains of chlortetracycline resistant strains were recovered in either hospitalized patients or hospital personnel.[17,30,33,35] There then followed an increase in the number of more resistant strains until 1953, when 78 per cent of the strains found in hospitalized patients and 64 per cent found in members of a hospital staff were resistant to this antibiotic.[23] On the other hand, the incidence of chlortetracycline resistant strains has not exhibited a similar increase in outpatients. The range of resistant strains in outpatients has been from 2 per cent to 19 per cent.[23,28,34]

TABLE III
INCIDENCE OF CHLORTETRACYCLINE RESISTANT STRAINS OF MICROCOCCUS PYOGENES

Year	Authors	Country	Per cent Resistant
	a. Hospitalized Patients		
1949	Schneierson[33]	U. S. A.	0.0
1949	Nichols and Needham[17]	U. S. A.	0.0
1950	Spink[18]	U. S. A.	60.0
1950	Schneierson[33]	U. S. A.	4.5
1951	Schneierson[33]	U. S. A.	20.0
1952	Finland and Haight[21]	U. S. A.	25.0
1953	Miyahara et al.[24]	U. S. A.	21.0
1953	Dowling et al.[23]	U. S. A.	78.0
	b. Outpatients		
1951	Anderson[34]	England	19.0
1952	Birnstingle et al.[28]	England	4.0
1953	Dowling et al.[23]	U. S. A.	2–12
	c. Hospital Staff		
1949	Needham and Nichols[35]	U. S. A.	0.0
1949	Rice and Lonargan[30]	U. S. A.	0.0
1952	Clark et al.[36]	England	60.0
1953	Dowling et al.[23]	U. S. A.	64.0

The incidence of antibiotic resistant strains of M. pyogenes isolated from patients at the Minneapolis General Hospital has been investigated for the years 1951 through 1953.[37] A summary of the strains of staphylococci which were found to be resistant *in vitro* to over 50 units or μg. of antibiotic per milliliter is found in Table IV. Sixty-two and five-tenths per cent of 48 strains studied in 1951; 57.2 per cent of forty-two strains in 1952; and 67.7 per cent of 153 strains in 1953 were not inhibited by 50 units of penicillin per milliliter. Streptomycin resistant strains increased from 48 per cent in 1951 to 65.3 per cent in 1953. With oxytetracycline (terramycin®) there was an increase from 38 per cent in 1951 to 47.5 per cent in 1952 and to 62.7 per cent in 1953. Results with chlortetracycline (aureomycin) have been similar to those with oxytetracycline.

TABLE IV
COMPARISON OF STRAINS OF MICROCOCCUS PYOGENES ISOLATED AT MINNEAPOLIS GENERAL HOSPITAL*

Antibiotic	Year	Total Strains Tested	No. Resistant	Percentage
Penicillin	1951	48	30	62.5
	1952	42	24	57.2
	1953	153	96	62.7
Streptomycin	1951	48	23	48.0
	1952	41	20	48.8
	1953	153	100	65.3
Oxytetracycline	1951	42	16	38.0
	1952	40	19	47.5
	1953	153	96	62.7
Chlortetracycline	1951	48	11	23.0
	1952	42	14	33.2
	1953	153	96	62.7
Chloramphenicol	1951	48	12	25.0
	1952	40	1	2.5
	1953	153	1	0.65

* These strains were resistant to 50 units or μg. of antibiotic per milliliter.

The data in regard to chloramphenicol (chloromycetin®) are quite different from those of the other antibiotics. There has been a decrease in the number of chloramphenicol resistant strains since 1951. This decrease was associated with a reduction in the use of this antibiotic in 1951. The number of strains which were resistant to 50 μg. per milliliter decreased from 25 per cent in 1951 to 2.5 per cent in 1952 and to 0.65 per cent in 1953. Of all strains of staphylococci which were studied at the Minneapolis General Hospital in 1953, 95 per cent were inhibited in growth by 12.5 μg. per milliliter.

A summary of the strains which were resistant to 3.1 μg. per milliliter of the other antibiotics is found in Table v. Approximately 10 per cent were found to be moderately resistant to bacitracin each year. There has been a rising number of strains resistant to erythromycin.

TABLE V
COMPARISON OF STRAINS OF MICROCOCCUS PYOGENES ISOLATED AT MINNEAPOLIS GENERAL HOSPITAL*

Antibiotic	Year	Total Strains Tested	No. Resistant	Percentage
Bacitracin	1951	20	2	10
	1952	26	3	11.5
	1953	153	12	7.8
Neomycin	1951	12	0	0.0
	1952	24	6	25.0
	1953	153	7	4.5
Erythromycin	1952	30	0	0.0
	1953	153	32	21.0
Carbomycin	1953	153	32	21.0

* These strains were resistant to 3.1 units or μg. of antibiotic per milliliter.

In 1952 all of the thirty strains studied at the Minneapolis General Hospital were sensitive to 3.1 μg. of erythromycin per milliliter. But in 1953, 21 per cent of 153 strains examined were observed to be resistant to this concentration. The majority of these resistant strains required from 200 to 400 μg. of erythromycin per milliliter for inhibition of growth. Carbomycin has not been used extensively but every strain of staphylococcus that has exhibited resistance to erythromycin in this study has also been resistant to carbomycin in similar concentrations. Cross resistance is evident between these two antibiotics.

Thus it becomes apparent from studies carried out during the last decade at the University of Minnesota and elsewhere that the incidence of antibiotic resistant strains of staphylococci is directly related to the quantities of antibiotics being administered to a hospital population. As each antibiotic is extensively employed in therapy, the incidence of resistant strains of staphylococci rises for that antibiotic. If the use of an antibiotic is curtailed, as with chloramphenicol, the incidence drops. This correlation of antibiotic resistance with the quantities of antibiotic being used is unique for staphylococcus only and is particularly applicable to hospital populations in large centers.

EPIDEMIOLOGY OF STAPHYLOCOCCAL INFECTIONS

A knowledge of the mechanisms involved in the spread of these bacteria is important because prevention of infection by these highly resistant strains must be attempted. The primary reservoir of pathogenic staphylococci is the human nasopharynx. The carrier rate of coagulase positive staphylococci in the general population has been reported to be 25 per cent to 50 per cent, whereas in hospital personnel the carrier rate is reported to be 50 per cent to 75 per cent.[22,25,31,38,39] Nasal carriers are usually skin carriers of the same strains.[39,41]

The mechanism by which staphylococci invade wounds from nasal carriers is not clearly understood. It has been reported that contamination of the air with staphylococci in the dust from the clothing and bed clothes of carriers is of more significance than the transfer of staphylococci by droplets from the nasopharynx.[42,43] Surgical wounds may be contaminated by surgeons when small puncture holes of the gloves permit accumulated perspiration containing staphylococci to ooze into the tissues.[44]

The incidence of antibiotic resistant strains of staphylococci found in hospitalized patients is roughly proportional to the duration of time spent in the hospital. This was reported by Cairnes and Summers,[19] who found a group of hospitalized patients to possess antibiotic resistant strains in 25 per cent of the cases on the first day, 52 per cent from the second to the seventh day and 68 per cent after the eighth day. Rountree and Barbour[31] studied the carrier rate of 127 nurses during the first sixteen weeks of training and observed an increase in the carrier rate from 52.6 per cent to 71.4 per cent, and in the penicillin resistant carrier rate from 4.3 per cent to 32.1 per cent. Lepper et al.[45] reported an increase from 0 to 75 per cent of erythromycin resistant staphylococci among members of a hospital staff during a five-month period when the drug was employed frequently in treatment of patients. The incidence of infected surgical wounds has been recently reported by Howe[46] to be increasing; he stated that the rate of infection of clean wounds after surgery gradually increased from 1.24 per cent

in 1949 to 4.66 per cent in 1953 at the Massachusetts Memorial Hospitals despite the prophylactic use of antibiotics. This was attributed to an abnormally high penicillin resistant carrier rate in the hospital personnel and patients.

Bacteriophage typing of staphylococci has revealed a predominance of group III. Bacteriophage patterns of staphylococci isolated from infections caused by antibiotic resistant strains encountered in hospitals indicate the selection of a specific group of staphylococci having the property of resistance.[47–49] In a study to be reported elsewhere bacteriophage typing was used in the identification of strains of staphylococci isolated from twenty-three hospitalized patients who developed infections from erythromycin resistant staphylococci.[50] In three of the patients the resistant strains isolated before and after therapy were of the same bacteriophage types, although the pretherapy strains were sensitive to erythromycin. This indicated that selective action of erythromycin took place in the patient. In the other twenty patients bacteriophage typing suggested that erythromycin resistant strains appeared as a result of cross infections.

METHODS

Cultures for staphylococci were obtained from nasopharynges of 208 persons on the surgical service of the University of Minnesota Hospitals in January 1954.* The group consisted of physicians, nurses, nurses' aids and orderlies. During the same month similar cultures were obtained from 200 persons in the outpatient clinic who had not been hospitalized or treated with antibiotics for at least the preceding six months. A third group consisted of ninety-six strains isolated from sixty-six inpatients on the surgical service who had infections; these strains were collected from July 1, 1953 to January 1954.

All cultures were inoculated on human blood agar and trypticase soy or thioglycolate broth, and isolates were made from these media. All cultures of staphylococci were examined for coagulase activity by employing human plasma with the slide and test tube technics.

The technic described by Waisbren[51] employing serial dilutions of antibiotics in broth was used to test the *in vitro* susceptibility of all strains of coagulase positive staphylococci to antibiotics. Susceptibility was determined for each strain with the following antibiotics: penicillin, streptomycin, chlortetracycline, oxytetracycline, chloramphenicol, bacitracin and erythromycin.

* The cooperation of Dr. O. H. Wangensteen and his staff in this study is appreciated.

All cultures were studied for bacteriophage lysis as a typing procedure. The method of bacteriophage typing described by Williams and Rippon[52] and Blair and Carr[53] was used. This method consisted of placing each of thirty-four different bacteriophages in specified dilutions on an agar plate which had been heavily inoculated with a culture. If a strain was not lysed with any of the diluted bacteriophages, it was re-examined with undiluted bacteriophages. The pattern of lysis obtained determined the type of strain being examined. The types were divided into groups according to the following classification:* group I, bacteriophages 29, 52, 52A, 79; group II, bacteriophages 3A, 3B, 3C, 55; group III, bacteriophages 6, 7, 42E, 47, 53, 54, 70, 73, 75, 77; and group IV, bacteriophage 42D. Strains of staphylococci were designated as members of groups according to the pattern of lysis. In some cases overlapping occurred to such a degree that strains were designated as intermediate members of groups.

TABLE VI
STAPHYLOCOCCI ISOLATED FROM HOSPITAL PERSONNEL AND OUTPATIENTS

Group	Number of Persons	Total Strains		Coagulase Positive Strains		
		No.	Per cent	Total	Per cent of Persons	Per cent of Strains
Hospital personnel	208	178	85.6	68	32.7	38.2
Outpatients	200	148	74.0	41	20.5	27.7

RESULTS

Staphylococci were isolated from 85.6 per cent of the members of the hospital staff and 74 per cent of the group in the outpatient department. Coagulase positive staphylococci were obtained from sixty-eight (32.7 per cent) of the hospital staff and forty-one (20.5 per cent) of the outpatients. These data are presented in Table VI.

* This classification was suggested in January 1954 by the subcommittee on Bacteriophage Typing of Staphylococci of the International Committee on Bacteriological Nomenclature and employs nineteen strains of bacteriophage.[54]

The results of tests for *in vitro* susceptibility of all coagulase positive strains from the three environmental groups are compared in Table VII.

Penicillin. Approximately 41 per cent of the strains obtained from the hospital staff and from the infections of patients were resistant to 1,000 units of penicillin per milliliter, and only 19.1 and 5.2 per cent respectively were sensitive to 1.0 unit per milliliter. In contrast to this high degree of resistance, 60.9 per cent of the strains from the outpatients were sensitive to 1.0 unit per milliliter.

TABLE VII

RESULTS IN PERCENTAGE OF *in vitro* SUSCEPTIBILITY OF MICROCOCCUS PYOGENES (ISOLATED FROM DIFFERENT GROUPS) TO ANTIBIOTICS IN UNITS OR MICROGRAMS PER MILLILITER

Group	0.1–1.0	1.0–10	10–100	100–500	500–1000	>1000
Penicillin (units per ml.)						
Staff	19.1	2.9	29.4	7.3	...	41.1
Inpatients	5.2	3.1	8.3	33.3	9.3	40.6
Outpatients	60.9	7.3	21.9	9.7	...	....
Streptomycin (μg. per ml.)						
Staff	.....	25.0	26.4	2.9	...	45.5
Inpatients	.....	7.2	13.5	1.0	...	78.9
Outpatients	.....	70.7	29.3	....	...	....
Chlortetracycline (μg. per ml.)						
Staff	54.4	7.3	19.1	19.1	...	....
Inpatients	3.1	21.8	70.8	4.1	...	....
Outpatients	85.3	14.7	....	....	...	....
Oxytetracycline (μg. per ml.)						
Staff	47.0	14.7	10.2	27.9	...	....
Inpatients	8.3	13.5	46.8	30.2	1.0	....
Outpatients	75.6	24.4	....	....	...	....
Chloramphenicol (μg. per ml.)						
Staff	.....	92.6	7.3	....	...	....
Inpatients	23.9	71.5	3.1	2.1	...	....
Outpatients	.....	97.5	2.5	....	...	....
Bacitracin (Units per ml.)						
Staff	32.3	67.7	....	....	...	....
Inpatients	22.9	73.9	3.1	....	...	....
Outpatients	9.4	90.6	....	....	...	....
Erythromycin (μg. per ml.)						
Staff	97.0	....	....	....	1.5	1.5
Inpatients	91.6	1.1	1.1	1.1	3.1	2.0
Outpatients	100.0	....	....	....	...	....

Streptomycin. The greatest degree of resistance to streptomycin was demonstrated by strains isolated from infections in hospitalized patients. Approximately 79 per cent of these strains were not inhibited by 1,000.0 μg. per milliliter. Of the strains from the hospital staff 45.5 per cent were resistant to 1,000.0 μg. per milliliter. All strains from the outpatients were inhibited in a range of 1.0 to 100.0 μg. per milliliter, with 70.7 per cent occurring in a therapeutic range of 1.0 to 10 μg. per milliliter.

Chlortetracycline (Aureomycin). Staphylococci isolated from the septic infections of hospitalized patients revealed the highest incidence of resistance, since 75 per cent required 10.0 to 500.0 μg. per milliliter for inhibition. Of the strains from the hospital staff, 54.4 per cent were susceptible to 0.1 to 1.0 μg. per milliliter.

Oxytetracycline (Terramycin). The results with oxytetracycline were similar to those obtained with chlortetracycline. Of the strains from inpatients, 77 per cent required 10 to 500 μg. per milliliter for inhibition of growth; 47 per cent of the strains from the hospital personnel were sensitive in a range of 0.1 to 1.0 μg. per milliliter, and 39 per cent required 10 to 500 μg. per milliliter for inhibition. Strains which were most sensitive were from the outpatients, 75.6 per cent being sensitive to 0.1 to 1.0 μg. per milliliter, and all strains were susceptible to 10.0 μg. per milliliter.

Chloramphenicol (Chloromycetin). This antibiotic has been used to a limited degree at the University of Minnesota Hospitals. This is reflected in the similarity of results in all three environmental groups. Over 90 per cent of the strains from the hospital staff and the outpatients were susceptible to 1.0 to 10.0 μg. per milliliter. In the inpatient group 23.9 per cent were susceptible to 0.1 to 1.0 μg. per milliliter.

Bacitracin. This antibiotic has been used parenterally and topically in a few cases of infections in the University of Minnesota Hospitals. Results in each group were similar in that all strains were inhibited in a range of 0.1 to 10.0 μg. per milliliter with the exception of 3.1 per cent of the strains obtained from infections of inpatients that required 10.0 to 100.0 μg. per milliliter for inhibition.

Erythromycin (Ilotycin or Erythrocin). A high incidence of susceptibility of strains from all groups was exhibited for erythromycin. One hundred per cent of the strains from outpatients, 97 per cent from the hospital staff and 91.6

per cent from infections of patients were inhibited by 0.1 to 1.0 μg. per milliliter. Approximately 3 per cent and 5 per cent of the strains from the staff and inpatients, respectively, required over 500.0 μg. per milliliter for inhibition of growth.

TABLE VIII
INCIDENCE OF PHAGE GROUPS OF STAPHYLOCOCCI ISOLATED FROM DIFFERENT ENVIRONMENTAL GROUPS

Bacteriophage Group	Staff (%)	Inpatients (%)	Outpatients (%)
I	7.3	1.0	17.0
II	8.7	2.0	21.9
III	52.9	56.2	19.5
IV	....	....	2.4
I and III	5.9	....	9.7
I and IV	1.4	....	....
Non-typable	23.5	40.6	29.2

BACTERIOPHAGE TYPES OF STAPHYLOCOCCI ISOLATED FROM DIFFERENT ENVIRONMENTAL GROUPS

As can be seen in Table VIII, the strains of staphylococci isolated from outpatients were distributed throughout all groups. In contrast, there was a predominance of group III and non-typable strains among the cultures obtained from the members of the hospital staff and the inpatients. Group III and the non-typable group contained the majority of strains having increased antibiotic resistance. The number of strains in groups I and II from the hospital staff and inpatients was much less than the percentage of strains in these groups in the outpatients. These findings are in agreement with previous reports that most antibiotic resistant strains of staphylococci are in bacteriophage group III.

COMMENTS

It is evident from other published reports, from the findings at the University of Minnesota Hospitals and at the Minneapolis General Hospital, that strains of staphylococci recovered from persons outside a hospital or who have not received an antibiotic differ from those obtained from patients in a hospital and from hospital personnel. A high percentage of the former are susceptible to the action of antibiotics whereas "hospital" strains possess considerable resistance to most of the antibiotics. Bacteriophage typing confirms the finding that the appearance of resistant staphylococci is a property of certain strains, occurring most frequently among the group III bacteriophage types and the group that did not show lysis. Resistance to each antibiotic is roughly correlated with the quantities of antibiotics used.

It can be concluded that antibiotic resistant strains predominate in the hospital environment as a result of a selective process of antibiotic action. These resistant strains are carried by patients and by members of the hospital personnel. Highly resistant strains infect wounds, cause pneumonia, genitourinary tract infections and, in some cases, septicemia ensues with often fatal results.

A great need exists for an awareness of the nature and magnitude of this problem. The ubiquity of the pathogenic antibiotic resistant strains of staphylococci in the hospital environment indicates the need for prophylactic measures to prevent cross infections of patients and the establishment of the carrier state in members of the hospital staff.

CONCLUSIONS

1. There has been an increasing incidence of antibiotic resistant strains of staphylococci in the nasopharynges of persons who are closely associated with the environment of hospitals and in septic infections which occur in patients during hospitalization.

2. The incidence of antibiotic resistance of strains is roughly proportional to the quantity of an antibiotic used in a hospital.

3. The incidence of antibiotic resistant strains in the general population that is not associated with hospitals is much less than in hospital personnel.

4. Infections with antibiotic resistant strains occur as a result of two mechanisms: (1) the emergence of resistant strains from more sensitive strains by a given antibiotic selecting resistant cocci, thus permitting their survival and multiplication, and (2) cross infection by strains which are previously resistant to antibiotics.

5. Antibiotic resistance is a property of certain strains of staphylococci, the majority of these strains being in bacteriophage group III and a group which did not show lysis.

6. Vigilance in aseptic technic to prevent cross infections of patients and the carrier state in the hospital personnel is necessary as measures of prevention.

REFERENCES

1. Spink, W. W., Ferris, V. and Vivino, J. J. Comparative *in vitro* resistance of staphylococci to penicillin and to sodium sulfathiazole. *Proc. Soc. Exper. Biol. & Med.*, 55: 207, 1944.
2. North, E. A. and Christie, R. Observations on sensitivity of staphylococci to penicillin. *M. J. Australia*, 2: 44, 1945.
3. Rammelkamp, C. H. and Maxon, T. Resistance of Staphylococcus aureus to action of penicillin. *Proc. Soc. Exper. Biol. & Med.*, 51: 386, 1942.
4. Rantz, L. A. and Kirby, W. M. M. Action of penicillin on staphylococcus *in vitro*. *J. Immunol.*, 48: 335, 1944.
5. Bondi, A. and Dietz, C. C. Penicillin resistant staphylococci. *Proc. Soc. Exper. Biol. & Med.*, 60: 55, 1945.
6. Gallardo, E. Sensitivity of bacteria from infected wounds to penicillin. *War Med.*, 7: 100, 1945.
7. Plough, H. H. Penicillin resistance of Staphylococcus aureus and its clinical implications. *Am. J. Clin. Path.*, 15: 446, 1945.
8. Böe, J. and Vogelsang, T. M. Penicillin-resistant pathogenic staphylococcus: increasing incidence of resistant strains in the upper respiratory tract. *Acta path. et microbiol. Scandinav.*, 29: 368, 1951.
9. Blair, J. E., Carr, M. and Buchman, J. Action of penicillin on staphylococci. *J. Immunol.*, 52: 281, 1946.
10. Barber, M. and Whitehead, J. E. M. Bacteriophage types in penicillin-resistant staphylococcal infection. *Brit. M. J.*, 2: 565, 1949.
11. Summers, G. A. C. Penicillin-resistant staphylococci—distribution among out patients. *Lancet*, 1: 135, 1952.
12. Beigelman, P. M. and Rantz, L. A. Clinical importance of coagulase-positive penicillin-resistant Staphylococcus aureus. *New England J. Med.*, 242: 353, 1950.
13. Forbes, G. B. Infection with penicillin-resistant staphylococci in hospitals and general practice. *Brit. M. J.*, 2: 569, 1949.
14. Martyn, G. Staphylococci in the newborn—their coagulase production and resistance to penicillin and streptomycin. *Brit. M. J.*, 1: 710, 1949.
15. Rountree, P. M. and Thomson, E. F. Incidence of penicillin-resistant and streptomycin-resistant staphylococci in a hospital. *Lancet*, 2: 501, 1949.
16. Berger, K. Über die Häufigkeitszunahme penicillinresistente pathogener Kokken. *Wien. med. Wchnschr.*, 99: 536, 1949.
17. Nichols, D. R. and Needham, G. M. Aureomycin in the treatment of penicillin–resistant staphylococcic bacteremia. *Proc. Staff Meet. Mayo Clin.*, 24: 309, 1949.
18. Spink, W. W. Clinical and biologic significance of penicillin-resistant staphylococci including observations with streptomycin, aureomycin, chloramphenicol and terramycin. *J. Lab. & Clin. Med.*, 37: 278, 1951.
19. Cairnes, H. J. F. and Summers, G. A. C. Penicillin-resistant staphylococci—incidence in relation to length of hospital stay. *Lancet*, 1: 446, 1950.
20. Rountree, P. M. Cross-infection of surgical wounds. *M. J. Australia*, 2: 766, 1951.
21. Finland, M. and Haight, T. H. Antibiotic-resistance of pathogenic staphylococci. *Arch. Int. Med.*, 91: 143, 1953.
22. Rountree, P. M. and Thomson, E. F. Incidence of antibiotic-resistant staphylococci in a hospital. *Lancet*, 2: 262, 1952.
23. Dowling, H. F., Lepper, M. H. and Jackson, G. G. Observations on the epidemiological spread of antibiotic-resistant staphylococci with measurements of the changes in sensitivity to penicillin and aureomycin. *Am. J. Pub. Health*, 43: 860, 1953.
24. Miyahara, B. T., Cariker, K. and Clapper, W. E. Cross-resistance in staphylococci and the effect of combinations of antibiotics on resistant strains. *J. Lab. & Clin. Med.*, 41: 550, 1953.
25. Barber, M., Hayhoe, F. and Whitehead, J. E. M. Penicillin-resistant staphylococcal infection in a maternity hospital. *Lancet*, 2: 1120, 1949.
26. Thompson, B. A. and Schwabacher, H. The incidence of penicillin-resistant staphylococci in a semi-closed community. *J. Clin. Path.*, 4: 350, 1951.
27. Vogelsang, T. M. The incidence of penicillin-resistant pathogenic staphylococci isolated from the upper respiratory tracts of young healthy persons. *Acta path. et microbiol. Scandinav.*, 29: 363, 1951.
28. Birnstingle, M. A., Shooter, R. A. and Hunt, M. F. Sensitivity to five antibiotics of strains of Staphylococcus pyogenes isolated from out patients. *Brit. M. J.*, 2: 253, 1952.
29. Linsell, W. D. The antibiotic sensitivity of pathogenic staphylococci. *J. Clin. Path.*, 5: 165, 1952.
30. Rice, W. G. and Lonargan, A. M. Penicillin-resistant staphylococci. *Am. J. Clin. Path.*, 19: 1083, 1949.
31. Rountree, P. M. and Barbour, R. G. H. Nasal carriers rates of Staphylococcus pyogenes in hospital nurses. *J. Path. & Bact.*, 63: 313, 1951.
32. Barber, M. and Rozwodowski-Dowzenko, M. Infection by penicillin-resistant staphylococci. *Lancet*, 2: 641, 1948.
33. Schneierson, S. S. Changes in bacterial sensitivity to aureomycin and chloramphenicol in the course of the past three years. *J. Lab. & Clin. Med.*, 40: 48, 1952.
34. Anderson, K. The aureomycin sensitivity of 100 pathogenic strains of Staphylococcus aureus. *J. Clin. Path.*, 4: 355, 1951.
35. Needham, G. M. and Nichols, D. R. Recent changes in sensitivity of Micrococcus pyogenes to various antibiotic agents. *J. Lab. & Clin. Med.*, 41: 150, 1953.
36. Clark, S. K. R., Dalgleish, P. G., Gillespie, W. A. Hospital cross infections with staphylococci resistant to several antibiotics. *Lancet*, 1: 1132, 1952.
37. Wise, R. I. Staphylococcal sepsis: Control with antibiotics. *Minnesota Med.*, 37: 857, 1954.
38. McFarlan, A. M. Incidence of pathogenic staphylococci in the nose. *Brit. M. J.*, 2: 939, 1938.
39. Miles, F. A., Williams, R. E. O. and Clayton-Cooper, B. The carriage of Staphylococcus aureus in man and its relation to wound infection. *J. Path. & Bact.*, 56: 513, 1944.

40. Allison, V. D. and Hobbs, B. C. An inquiry into the epidemiology of pemphigus neonatorum. *Brit. M. J.*, 2: 1, 1947.
41. Williams, R. E. O. Skin and nose carriage of bacteriophage types of Staphylococcus aureus. *J. Path. & Bact.*, 58: 259, 1946.
42. Duquid, J. P. and Wallace, A. T. Air infection with dust liberated from clothing. *Lancet*, 2: 845, 1948.
43. Hare, R. and MacKenzie, D. M. The source and transmission of nasopharyngeal infections due to certain bacteria and viruses. *Brit. M. J.*, 1: 865, 1946.
44. Devenish, E. A. and Miles, A. A. Control of Staphylococcus aureus in an operating theatre. *Lancet*, 1: 1088, 1939.
45. Lepper, M. H., Moulton, B., Dowling, H. F., Jackson, G. G. and Kofman, S. Epidemiology of erythromycin-resistant staphylococci in a hospital population—effect on therapeutic activity of erythromycin, p. 308. Antibiotics Annual, 1953–1954.
46. Howe, C. W. Postoperative wound infections due to Staphylococcus aureus. *New England J. Med.*, 251: 411, 1954.
47. Rountree, P. M., Barbour, R. G. H. and Thomson, E. F. Incidence of penicillin-resistant and streptomycin-resistant staphylococci in a hospital. *Lancet*, 1: 435, 1951.
48. Roereg, R. N., Metzer, J. F., Fusillo, M. H. and Ernst, K. F. Phage typing of antibiotic-resistant staphylococci. II. Phage types of organisms isolated from various sources. p. 329. Antibiotics Annual 1953–1954.
49. Knight, V. and Holzer, A. R. Studies on staphylococci from hospital patients I. Predominance of strains of Group III phage patterns which are resistant to multiple antibiotics. *J. Clin. Investigation*, 33: 1190, 1954.
50. Wise, R. I., Voigt, A. E., Collin, M. V. and Cranny, C. L. Origin of erythromycin-resistant strains of Micrococcus pyogenes in infections—bacteriophage types and *in vitro* resistance of cultures to antibiotics. *Arch. Int. Med.*, 95: 419, 1955.
51. Waisbren, B. A., Carr, C. and Dunnette, J. The tube dilution method of determining bacterial sensitivity to antibiotics. *Am. J. Clin. Path.*, 21: 884, 1951.
52. Williams, R. E. O. and Rippon, J. E. Bacteriophage typing of Staphylococcus aureus. *J. Hyg.*, 50: 320, 1952.
53. Blair, J. E. and Carr, M. The bacteriophage typing of staphylococci. *J. Infect. Dis.*, 93: 1, 1953.
54. Blair, J. E. Personal communication.

Allergic Reactions in Sites Recurrently Infected with Hemolytic Streptococcus*

FRANKLIN A. STEVENS, M.D.
New York, New York

IN 1927 and 1928 Amoss et al.[1] and Birkhaug[2,3] treated recurrent erysipelas with filtrates of cultures of hemolytic streptococci. This therapy was based on the then current assumption that these infections were caused by a single type of streptococcus producing a specific toxin and that adequate circulating antitoxin would prevent reinfections. These premises were incorrect; neither the toxin nor the streptococci recovered from these infections are specific,[4,5] and high blood levels of antitoxin have not prevented streptococcal infections.[6,7] Nevertheless the therapy was effective and recurrences were prevented.

Apart from the therapeutic value of these filtrates these investigators observed two clinical reactions of importance. First, within a few hours after inoculation the recurrently infected sites often became inflamed and, second, similar reactions followed streptococcal infections in distant locations. They believed that these sites were highly sensitized and that the inflammation was an allergic reaction to bacterial products absorbed from the inoculations of filtrate or from the infections. These clinical observations have been confirmed.[8] However, filtrates devoid of erythemagenic toxin are as effective therapeutically as those which contain toxin, and focal inflammatory reactions have been observed following inoculations of streptococcal "nucleoprotein."[8]

The results of the present investigation are reported in two sections: first, a study of this focal sensitization by direct testing and, second, studies of the bacteriology of the allergic reactions incited by distant infections.

COMPARISON OF NORMAL SKIN AND RECURRENTLY INFECTED SITES (TABLE I)

Clinical Material. Tests with streptococcal nucleoprotein were made in four patients who had recurrent streptococcal infections. Three had had repeated attacks on the same arm from paronychial infections. In the fourth, infections on one leg had been caused by a recurrent ulcer over the malleolus. Tests were performed following acute infections in sites which had been reinfected after intervals of from twelve to twenty-four months. In this interim old foci had apparently healed but were relighted when the last attack occurred. Neither edema nor fibrosis of the sites tested was present. Tests of edematous and fibrotic skin are too diffuse and faint to be read.

Bacteriologic Data. Beta hemolytic streptococci had been cultured from the foci or infected tissues in all these patients during attacks. In three cases, T. S., J. G. and M. R., strains of different types had been recovered from the last two successive infections. (Table II.[9]) Strains from the fourth patient were not studied serologically.

Nucleoprotein Solutions. Group A, type 10 (Griffith) hemolytic streptococci grown eighteen hours in liquid medium were centrifuged. The bacteria were washed in acetone and ether, dried and ground to a fine powder. This powder was then extracted in a 2 per cent solution of $NaHCO_3$ and after centrifuging the protein was precipitated from the supernatant fluid by adding weak acetic acid. It was precipitated seven times. The final solution was sterilized by filtration, the N content determined by the Kjeldahl method and five solutions prepared containing 10.0, 1.0, 0.1, 0.01 and 0.001 mg. of N per 100 ml.

Methods of Testing. An effort was made to test at regular intervals, but tests were made whenever and as long as the patients were available. They were begun about the fourth week after onset of the last infections. The results have been tabulated in Table I and arranged

* From the Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital, New York, New York.

TABLE I*

COMPARISON OF SENSITIZATIONS IN REINFECTED AND NON-INFECTED AREAS

	Patient T. S.		Patient J. G.		Patient M. P.		Patient J. M.	
Location of Foci and Infections	Ulcer left leg, lymphangitis, cellulitis		Paronychia erysipelas, right arm		Paronychia erysipelas, left arm		Paronychia erysipelas, left arm	
Hemolytic Streptococci Recovered in Attacks	4th attack. Group A Type ? from focus		3rd attack. Type 4 from tissue		3rd attack. Type 9(?) from tissue		3rd attack. Type 19 from tissue	
Minimal Doses (μg. N) Causing Reactions								
Weeks after Onset of Infection of Tests	Infected Skin	Normal Skin	Infected Skin	Normal Skin	Infected Skin	Normal Skin	Infected Skin	Normal Skin
4	0.01	1.0	0.1	1.0	0.01	1.0	0.1	1.0
6	0.1	10.0	0.01	0.1	0.001	0.1	0.001	0.1
8	0.01	1.0	0.01	1.0	0.01	1.0	0.01	1.0
12	1.0	10.0†	....	.....	0.1	1.0	0.01	0.1
16	0.1	10.0	0.1	10.0	1.0	10.0†	.....	....
27	....	.....	1.0	10.0†	.....	.....	0.1	1.0
37	0.1	10.0†	0.1	.....	0.1	10.0	0.1	1.0†

* The levels of sensitization to the "nucleoprotein" of hemolytic streptococcus have been compared in two sites in each patient. A site on one arm or leg had been recurrently infected with streptococci while the site on the opposite extremity was normal. Both sites were tested at intervals following an acute streptococcal infection of the recurrently infected area. Five testing solutions were used. These solutions were of varying strengths, graduated so that the testing doses, 0.1 ml. of each solution, were equivalent respectively to 10.0, 1.0, 0.1, 0.01 and 0.001 μg. of N. No immediate or intermediate reactions were noted following intracutaneous injection of 0.1 ml. of these solutions. The delayed inflammatory reactions to the nucleoprotein were read the following day, and the smallest dose causing a reaction 1.5 to 2.0 cm. in diameter was considered an index of the level of sensitization. These minimal reactive doses have been tabulated so that the levels of sensitization in the infected and in the normal areas may be compared at successive intervals following the acute infection. The strains of streptococci were typed by slide agglutination and classified according to Griffith.

† No reaction to this dose.

chronologically according to the week after the infection at which time the tests were made. A number of isolated tests which could not be reasonably correlated as to time with those done on other patients have been omitted.

Three tests were performed on the recently infected skin and on the skin of the opposite arm or leg with solutions of intermediate strength. One-tenth ml. of these solutions, equivalent to doses of 1.0, 0.1 and 0.01 μg. of nucleoprotein N, were injected intradermally. The reactions were read the next day. The smallest dose causing an inflammatory reaction 1.5 to 2.0 cm. in diameter was taken as the index of local sensitization. Further tests with larger and smaller doses (10.0 μg. and 0.001 μg.) were occasionally necessary to establish the minimal reacting dose.

Results of Comparative Tests. The doses giving minimal reactions in the affected and normal skin have been tabulated. (Table I.) Those of the normal skin indicate the constitutional levels of sensitization resulting from the streptococcal infection and are compared with the doses showing the levels of focal sensitization in the recently infected sites. The smaller the dose required to give a satisfactory reaction the higher the level of sensitization. The recently infected sites have been more highly sensitized and have remained sensitive after sensitization of the normal skin could no longer be detected.

ALLERGIC REACTIONS IN SENSITIZED SITES (TABLE II)

The allergic reactions mentioned in the introduction have not been studied sufficiently

TABLE II*

BACTERIOLOGIC DATA ON ALLERGIC REACTIONS

Patient	Earlier Attacks†			Inciting Infection and Cultures of Foci	Interval before Reaction (hr.)	Allergic Reaction and Cultures of Tissue	Duration of Inflammation (days)
	Location	No.	Cultures of Foci				
M. M.	Right leg	3	Group A, type ?	.....	36	No growth, no foci	4
	Left leg	1	H. strep., untyped	H. strep., untyped	..	.....	7
J. L.	Right leg	2	Group A, type ?	.....	48	No growth, no foci	3
	Left leg	0		H. strep. type 16	..	.....	10
R. B.	Right leg	3	H. strep., type 2	H. strep., type 13	..	.....	18‡
	Left leg	2	H. strep., type 13	.....	72	No growth, no foci	3
D. B.	Right leg	4	H. strep.	.....	24	No growth, no foci	2
	Left leg	2	H. strep., type 23(?)	H. strep., type ?	..	.....	8

* These patients had recurrent attacks of streptococcal infection on both legs. The number of infections which had occurred previously on each leg, the types of streptococci isolated from the last infections prior to the infections, and reactions described in this table are given under "earlier attacks." Although isolated infections of only one leg occurred, if both legs had been infected previously, usually an infection on one leg was followed after one or two days by an attack on the other. In the reactions described here the type of streptococcus isolated from the focus of the initial infection has been given under "inciting infection." Next, the "interval" before inflammation was observed on the opposite leg, then the result of "tissue cultures" of this secondary inflammatory attack. In none of the cultures of the secondary reactions included in this table were streptococci found, nor were they seen in microscopic sections. Agglutination, typing and classification of streptococci is according to Griffith.

† Data on the last culture made are given. Strains from foci were usually very granular and difficult to type; only about half could be typed in available typing sera.

‡ Infection relapsed.

to exclude local infection beyond reasonable doubt. In the following patients careful studies of the bacteriology of the inflamed tissues have been undertaken and the sequence of clinical events leading to these reactions described.

Clinical Material. Six of seventeen patients with recurrent infections of the legs have had bilateral attacks. Four of these were carefully studied. Since the series of infections in these patients began before they were brought under observation, nothing is known about the initial foci, but in subsequent attacks patches of dermatitis or infections around the nails were the apparent sources of infection. These foci might heal and be relighted with each attack. From them, infection spread in either one of two ways: (1) through the deeper layers of the skin upward over the legs, or (2) through the lymphatics to involve the legs and lower thighs. Both the foci and the tissues were cultured when attacks recurred.

Bacteriologic Methods. With the following exception the methods have been described in a previous publication.[9] After cleansing the skin with soap, acetone and ether, two closely parallel incisions were made in the inflamed tissue and the narrow strip of tissue removed. Cultures were then made on plates, with serum which was obtained from the depths of the incision. The superficial layers of the fragment of skin were fixed, sectioned and stained while the deeper part was incubated in a liquid medium. The cultures and sections were examined for hemolytic streptococci.

Clinical and Bacteriologic Data. Although isolated attacks occurred on either leg, the infections were often bilateral. Then one followed the other after an interval of two or three days and, while the second attack might be severe, it was usually milder. Those which were severe were obviously infections. The infection spread through the tissues and the induration and inflammation lasted several days. Streptococci could be cultured from the foci and were found in cultures made from the inflamed area. While some of the milder attacks were also infections, there were others in which no overt foci was present and cultures of the tissues were sterile;

in these the inflammation was diffuse and lasted only two or three days. As far as could be determined no bacteria were present in the tissues of the inflamed areas. Since these attacks followed infections on the opposite leg, they were presumably allergic reactions in sites sensitized by previous infection. The clinical and bacteriologic data obtained in four of these allergic reactions have been assembled in Table II.

COMMENTS AND CONCLUSIONS

Following acute attacks in patients with recurrent streptococcal infections the sites repeatedly infected are more highly sensitized to streptococcal nucleoprotein than the normal skin of other areas. The affected sites are not only more highly sensitive but this focal sensitization persists much longer than the general sensitization following the acute infection. In an occasional patient the tissues are mildly sensitive many years after the last attack.[10] They are also subject to allergic inflammatory reactions following distant streptococcal infections.

This study of focal sensitization is part of an inquiry into the mechanism responsible for the susceptibility to reinfection of tissues which have once been infected. When streptococcal infections recur in the same anatomic site chronic foci may account for invasion of the same area in successive attacks but, if the intervals between infections are long and attacks are caused by different strains, reinfections probably are not due to chronic foci.[9] Under these circumstances it is possible that sensitization of the affected tissues may be a factor leading to this susceptibility and to the localization of the infection. Apart from this sensitization, no other difference has been found so far between these and other tissues. Tissues recurrently infected with Streptococcus hemolyticus have been specifically sensitized to this species because focal allergic reactions have followed distal inoculations with streptococcal but not with staphylococcal nucleoprotein.[8]

REFERENCES

1. Amoss, H. L., Hansen-Pruss, O. C. and Bliss, E. A. Erysipeloid reactions as an allergic response. *Tr. A. Am. Physicians*, 43: 259, 1928.
2. Birkhaug, K. E. Erysipelas: Immunization with soluble toxin from streptococcus erysipelatis against recurrent attacks of erysipelas. *J. A. M. A.*, 88: 885, 1927.
3. Birkhaug, K. E. Erysipelas: Bacterial allergy to streptococcus erysipelatis in recurrent erysipelas. *J. A. M. A.*, 90: 1997, 1928.
4. Williams, A. W. Relationship of streptococci causing erysipelas. *Am. J. Pub. Health*, 19: 1303, 1929.
5. Eagles, G. H. Further studies on streptococci from scarlatina, erysipelas, and puerperal fever. *Brit. J. Exper. Path.*, 7: 286, 1926.
6. Stevens, F. A. and Dochez, A. R. The occurrence of throat infection with streptococcus scarlatinae without a rash. *J. A. M. A.*, 88: 1110, 1926.
7. Idem. The epidemiology of scarlatinal throat infections sine exanthemata. *J. A. M. A.*, 87: 2137, 1926.
8. Stevens, F. A. Chronic infectional edema. *J. A. M. A.*, 100: 1754, 1933.
9. Stevens, F. A. The behaviour of local foci causing recurrent streptococcal infections of the skin, subcutaneous tissues and lymphatics. *Surg., Gynec. & Obst.*, 99: 268, 1954.
10. Stevens, F. A. Streptococcal infection of the fibroedema of Melkersson's syndrome. *J. A. M. A.*, 156: 223, 1954.

Blood Volume in Patients with Laennec's Cirrhosis of the Liver as Determined by Radioactive Chromium-Tagged Red Cells*

SEYMOUR EISENBERG, M.D.
with the technical assistance of Miss Mary Sue McCall
Dallas, Texas

CHANGES in blood volume incident to the development of Laennec's cirrhosis of the liver have never been clearly elucidated. It has been generally accepted that patients with cirrhosis have an increase in total blood volume, primarily as a result of an expansion of the plasma space. Perera,[1] Hiller, Huffman and Levey,[2] and Bateman, Shorr and Elgvin[3] have reported uniformly increased values for plasma volume in subjects with cirrhosis as determined by T-1824 dye. Hyde, Berlin, Parsons, Lawrence and Port,[4] employing P^{32}-labeled red cells as a reference, failed to find this consistent expansion of the plasma volume in twenty-eight subjects with cirrhosis; considerable variation in total blood volume and in plasma volume was encountered, only eight of the twenty-eight patients exhibiting significant increases in plasma volume.

Because the protein to which the T-1824 dye is bound penetrates freely into the ascitic accumulation and because the instability of the radiophosphorus red cell complex does not permit prolonged equilibration periods, a study of this problem by application of the radiochromium-labeled red cell technic was undertaken. The objections to the previous methods would not pertain, since the stability of the Cr^{51} red cell complex does not permit accession of the reference material into the plasma space, as in the case of P^{32}, or into the lymphatic space, as in the case of the T-1824 dye.

Ascites was present in nineteen of the twenty subjects studied, and in twelve of these repeat observations were possible after the ascites disappeared with clinical improvement. In this manner, comparisons between untreated and treated patients with cirrhosis were possible, as well as comparisons between patients with cirrhosis and a group of twenty normal subjects.

MATERIALS AND METHODS

Twenty patients with Laennec's cirrhosis were studied. History, physical examination and laboratory studies in all subjects were characteristic of advanced hepatocellular damage with portal obstruction. All of the patients were inveterate alcoholics with severe dietary inadequacies. Ascites was present in nineteen instances; eight subjects had experienced one or more episodes of profuse upper gastrointestinal bleeding, although patients who had bled as recently as four months prior to the study were not included in the series. The pertinent clinical information is summarized in Table I.

The initial determinations of blood volume were made within twenty-four hours after admission to the hospital. The patients were then placed on a salt-poor, high caloric, high protein diet, and complete bedrest. In twelve of the nineteen subjects with ascites, the ascitic fluid gradually disappeared, at which time repeat observations were made. In three subjects the ascites persisted despite prolonged medical care, necessitating intermittent paracenteses. Two patients died shortly after admission, and a third patient left the hospital against advice.

Blood Volume. Blood volume was measured by the method of Sterling and Gray[5] as modified in our laboratory.[6] The patients were at complete bedrest during the entire procedure. Sixty-five cc. of whole venous blood were separated by centrifugation. The red cells were incubated with gentle agitation at 37°C. for one hour with 200 μc. of Cr^{51} (Na_2 Cr^{51} 04). After three washings the labeled red cells were mixed with the original plasma, and 45 cc. of the reconstituted whole blood were injected intravenously.

* From the Department of Internal Medicine, University of Texas, Southwestern Medical School, and the Medical Service and Radioisotope Unit of the Veterans Administration Hospital, Dallas, Texas. Presented before the Annual Meeting, American Federation for Clinical Research, Atlantic City, New Jersey, 1955.

TABLE I

SUMMARY OF PERTINENT CLINICAL DATA IN TWENTY PATIENTS WITH LAENNEC'S CIRRHOSIS OF THE LIVER

Patient	Age	Duration	Ascites	Edema	Jaundice	Fever	Esophageal Varices	Large Spider Angiomas	Albumin (gm. %)	Cyanosis	Clubbing	Hemoglobin (gm. %)
L. R. M.	34	? 1 mo.	+	+	+	+	+	−	2.4	+	−	9.8
C. A. R.	54	2–3 yr.	+	+	−	+	+	−	2.5	−	−	8.1
C. W. W.	35	3 yr.	+	+	+	+	+	+	2.7	+	−	9.2
R. E. W.	49	5 yr.	+	+	+	+	+	+	2.6	+	+	13.0
Y. Y.	61	?	+	+	+	+	−	−	2.6	−	−	10.2
T. G. P.	40	5 mo.	+	+	+	−	−	−	4.1	−	−	14.6
E. G. F.	58	3 yr.	+	−	−	Slight	+	−	3.4	−	−	11.4
W. W.	49	1 yr.	+	+	+	Slight	+	−	2.0	−	−	12.4
R. M. G.	57	2 yr.	+	+	+	Slight	−	−	2.2	−	−	12.4
W. G.	62	2–3 wk.	+	+	Slight	Slight	+	−	2.8	−	−	12.1
L. F.	48	? 1 mo.	+	+	+	+	+	Few	3.5	−	+	10.7
W. A. H.	59	1 mo.	+	+	−	−	−	−	3.2	−	−	12.4
B. L. W.	43	3 yr.	+	+	+	+	+	Few	3.2	−	−	11.0
W. R. G.	47	3 wk.	+	+	−	Slight	−	−	2.5	−	−	15.0
B. S. A.	73	? 1 yr.	+	−	+	+	−	−	1.7	−	−	9.8
M. M.	51	6 mo.	+	−	+	+	−	−	3.2	−	−	11.0
B. C. S.	56	? 3 yr.	+	±	−	±	−	−	3.1	−	−	13.3
B. M. G.	38	10 days	+	−	+	+	−	−	3.4	−	+	8.4
H. B.	43	3 wk.	−	−	+	Slight	−	....	....	+	+	11.2
H. R. P.	48	2 wk.	+	−	0	+	−	....	2.5	−	−	14.2

The initial venous blood sample was withdrawn without stasis three hours later, and the blood volume was calculated by dividing the total counts injected by the counts per cubic centimeter of the three-hour sample. Whole blood samples were counted for fifteen minutes in a Texas Deep Well Counter. Circulating red cell mass was estimated by multiplying the total blood volume by the venous hematocrit. The plasma volume was the difference between the total blood volume and the red cell mass. Values expressed as cubic centimeters per kilogram were calculated on the basis of the ultimate edema-free, ascites-free weight in the seventeen patients in whom this "dry" state was attained; in three instances the patients died or left the hospital before the recession of ascites and edema, and in these patients calculations were made on the basis of each patients' estimate of his weight prior to the development of the salt-retention syndrome. In many of the subjects considerable increments in tissue weight undoubtedly occurred as the fluid accumulations were being delivered; this change in tissue weight was disregarded in the calculations.

The plasma volume was not measured directly, as such measurements depend upon the use of labeled protein as a reference substance. Since the protein thus labeled not only penetrates the lymphatic space but enters the ascitic cavity in large amounts as well, serious errors might be induced by the egress of reference material from the vascular tree. On the other hand, estimation of the plasma space from the red cell mass and venous hematocrit has been criticized because of alleged variations in the venous hematocrit to body hematocrit ratio. However, Chaplin, Mollison and Vetter[7] have shown that the venous hematocrit to total body hematocrit ratio remains constant over wide variations in hematocrit range; further, they indicate that the small vessel hematocrit would have to change profoundly to induce serious errors in the total blood volume and plasma volume values. Therefore, blood volume and plasma volume have been estimated from the red cell mass and venous hematocrit rather than from direct measurements of the plasma space.

Hematocrits. The packed cell volume was determined in triplicate using Wintrobe tubes and a centrifuge with a radius of 15 cm. from the spindle center to the bottom of the hematocrit tube. The tubes were spun for sixty minutes at 2,400 revolutions per minute (647 gravities). No correction factors were applied.

RESULTS

The results are summarized in Table II, which includes the mean values for the measured functions in the series of twenty normal subjects.

Blood Volume, Red Cell Mass, and Plasma Volume in Untreated and Treated Subjects with Laennec's Cirrhosis, Compared with Normal Subjects. As compared with normal subjects the blood volume in patients with portal cirrhosis prior to treatment was not significantly increased ($p > .05$), although the mean was 9 cc./kg. greater than the mean for the normal group. The red cell mass in untreated subjects was 28.4 cc./kg.; this did not differ significantly from the normal group ($p > .7$). The plasma volume was 46.5 cc./kg., which slightly but significantly exceeded the values for the normal group ($p < .05$).

TABLE II

BLOOD VOLUME, RED CELL MASS, PLASMA VOLUME AND HEMATOCRIT IN SUBJECTS WITH LAENNEC'S CIRRHOSIS OF THE LIVER

(Comparison with Normal Values and between Pretreatment and Post-treatment Values)

Patient	Blood Volume[1] (cc./kg.)	Blood Volume[2] (cc./kg.)	Red Cell Mass[1] (cc./kg.)	Red Cell Mass[2] (cc./kg.)	Plasma Volume[1] (cc./kg.)	Plasma Volume[2] (cc./kg.)	Hematocrit[1]	Hematocrit[2]
20 Normal Males								
Mean	66.3	.....	27.8	.....	38.6	..	.....	....
S.D.	8.67	.....	3.27	.....	6.41	..	.....	....
L. R. M.	97	.....	46	.....	51	..	47	....
C. A. R.	85	.....	24	.....	61	..	29	....
C. W. W.	145/150	.....	48/50	.....	97	..	33	....
R. E. W.	88	81	34	34	54	47	39	42
Y. Y.	68	79	27	31	41	48	40	39
T. G. P.	65/66	62	30/29	29	35	33	46	47
E. G. F.	58	.....	19	.....	39	..	33	....
W. W.	74	74/74	31	32/29	43	42	42	43
R. M. G.	68/67.5	63	26/25	25	42	43	38	40
W. G.	75/85	79*	28/29.5	31*	47/55	48*	38/35	39*
L. F.	74	81	26	26	48	55	35	33
W. A. H.	50	48	20	21	30	27	40	44
B. L. W.	65	71	23.5	22	41	49	36	31
W. R. G.	73.5	67	33	33	41	34	45	50
B. S. A.	62	.....	21	.....	41	..	35	....
M. M.	61	61	23	24	38	37	37	39
B. C. S.	56	50	24	25	32	25	44	49
B. M. G.	74	93	22	28	52	65	29.5	30
H. B.	.....	96	.....	32	.....	64	.....	33.5
H. R. P.	65	.....	31	.....	34	..	47	....
Mean	75	.....	28.4	.....	46.5	..	38	....
S.E.	4.6	.....	1.736	.....	3.337	..	.....	....
p.	$>.05$	.....	$>.7$	.....	$<.05$	..	.....	....

Note: superior figure 1 indicates prior to treatment; superior figure 2 indicates after recession of ascites.
* Following paracentesis.

Despite the fact that all of the patients had comparable ascites and edema, seven patients showed an increase in plasma volume and red cell mass whereas the remainder had normal values for these functions. Therefore, the group as a whole was examined to determine what clinical features may be responsible for these changes in plasma volume and red cell mass.

If esophageal varices and/or cyanosis constituted the criteria for division of the patients into two groups, as shown in Table III, distinct differences were encountered. In the ten subjects displaying neither of these features the values for blood volume, red cell mass and plasma volume were similar to the mean values for the normal group. On the other hand, the values for plasma volume and total blood volume in ten subjects with esophageal varices and/or cyanosis significantly exceeded the values for the normal group ($p < .02$). Comparison of the values in four cyanotic subjects with the normal group revealed striking increases in all of the measured functions; however, the differences were not statistically significant, perhaps as a result of inadequate sampling.

Comparisons of Blood Volume, Red Cell Mass and Plasma Volume before and after Treatment. Comparisons were made between the results in twelve untreated patients and the same patients following the delivery of ascites and edema. There were no significant changes in

blood volume ($p > .6$), red cell mass ($p > .2$) or plasma volume ($p > .3$) as clinical improvement occurred, although considerable variations in plasma volume were encountered. The patients with ascites and cyanosis behaved exactly as did the remainder of the group in showing no significant change in plasma volume or red cell mass following the recession of ascites and edema with treatment.

TABLE III

MEAN VALUES FOR BLOOD VOLUME, RED CELL MASS AND PLASMA VOLUME IN CIRRHOTIC SUBJECTS WITH AND WITHOUT ESOPHAGEAL VARICES AND/OR CYANOSIS COMPARED WITH NORMAL VALUES

	Blood Volume (cc./kg.)	Red Cell Mass (cc./kg.)	Plasma Volume (cc./kg.)
Ten Cirrhotic Subjects without Cyanosis or Esophageal Varices			
Mean	64.3	25.7	38.6
Standard error	2.3753	1.4189	.....
Probability	> .5	> .1	.....
Ten Cirrhotic Subjects with Cyanosis and/or Esophageal Varices			
Mean	86	31.2	54.5
Standard error	7.709	2.983	5.3829
Probability	< .02	> .3	< .01
Four Cirrhotic Subjects with Cyanosis			
Mean	106.5	40	66.5
Standard error	12.99	4.082	10.54
Probability	> .05	> .1	> .1

Changes in blood volume incident to paracentesis were measured in two subjects.

	Blood Volume (cc.)	Red Cell Mass (cc.)	Plasma Volume (cc.)	Hematocrit (mm.)
Patient W. G.				
Ascites	4,110	1,560	2,550	38
6 hr. after paracentesis*	4,330	1,690	2,640	39
Ascites 1 wk. after paracentesis	4,670	1,625	3,045	35
Patient B. W.				
Ascites	5,730	2,080	3,650	36.4
30 min. after paracentesis*	5,730	2,060	3,670	36
90 min. after paracentesis*	6,110	2,120	3,990	34.8

* There was no access to water for twelve hours following paracentesis.

In one subject measurements were made prior to treatment, after clinical improvement and delivery of ascites, and again after ascites had reappeared following the resumption of drinking and dietary neglect.

	Blood Volume (cc.)	Red Cell Mass (cc.)	Plasma Volume (cc.)	Hematocrit (mm.)
Patient T. P.				
Ascites	5,548	2,541	3,007	46
No ascites	5,270	2,460	2,810	47
Ascites	5,600	2,440	3,160	43.5

One subject was studied prior to treatment, after clinical improvement, and, ultimately, following the establishment of a portacaval shunt.

	Blood Volume (cc.)	Red Cell Mass (cc.)	Plasma Volume (cc.)	Hematocrit (mm.)
Patient W. W.				
Ascites	5,160	2,180	2,980	42
No ascites	5,200	2,250	2,950	43
After portacaval shunt	5,600	2,200	3,400	39

COMMENTS

The results of this study, taking the entire group of twenty patients as a whole, indicate that the plasma volume is significantly expanded in patients with Laennec's cirrhosis. This is in accord with the findings of Perera,[1] Hiller, Huffman and Levey,[2] and Bateman, Shorr and Elgvin.[3] The finding that this expansion of the plasma volume is an inconsistent feature in cirrhotic subjects is in accord with the findings of Hyde, Berlin, Parsons, Lawrence and Port.[4]

When the twenty patients were divided according to the presence or absence of esophageal varices and/or cyanosis, significant differences between the two groups were encountered. Subjects without these features had normal values for blood and plasma volume whereas in subjects with esophageal varices or cyanosis the blood volume and plasma volume were significantly increased. This would suggest that the plasma volume expansion which was encountered in some subjects was not related to the presence of ascites or edema but to the stimulus for erythropoiesis in cyanotic subjects

and to the increase in the capacity of the vascular tree incident to the development of an extensive system of collaterals. That hypervolemia may be a passive consequence of an expanded vascular bed is in accord with the findings of Perera[1] and is similar to the observation that expansion of the blood volume in patients with congestive heart failure is primarily due to the increase in the capacity of the central portion of the vascular system, i.e., heart, great vessels and pulmonary vascular tree.[8]

The occurrence of arterial oxygen unsaturation in patients with advanced portal cirrhosis was noted by Keyes and Snell[9] and by Wilson, Ebert, Borden, Pearson, Johnson, Falk and Dempsey.[10] The latter group found the mean oxygen saturation of arterial blood to be 94.4 $\pm$ 3.9 per cent in ten unselected patients with cirrhosis; by the method of Berggren[11] they adduced evidence of a pulmonary arteriovenous shunt some five times that found in a group of normal persons. If, for reasons yet to be elucidated, significant pulmonary arteriovenous communications do develop in all or some patients with portal cirrhosis, polycythemia secondary to the decrease in arterial oxygen saturation might eventuate. In the four cyanotic subjects* in the present series the mean value for red cell mass was 40 cc./kg., which greatly exceeded the range of values in the normal group (20 to 32 cc./kg.). In patient H. B. the arterial oxygen saturation was determined to be 89 per cent and in patient R. E. W. it was 88 per cent; in patient H. B. there was a 19 per cent pulmonary arteriovenous shunt by the method of Berggren.[11]

A decrease in hemoglobin concentration and red blood cell count, in the absence of bleeding, is a well documented clinical observation in patients with cirrhosis of the liver; however, the cause of this apparent anemia has never been clearly elucidated. Two alternative explanations for the low hemoglobin concentration and red cell count have been proposed: first, the fact that a decrease in these values actually represents a true anemia (defined as a diminished red cell mass) has been challenged, the low values being ascribed instead to an expansion of the plasma volume;[3] second, several hematologic mechanisms have been proposed to explain the development of an anemia in these subjects.

* Patients C. W., L. M., R. E. W., H. B.

Decreased red cell production, secondary either to severe malnutrition or to a deficiency of one of the specific maturation factors, has been suggested as a frequent causative mechanism. Support is lent this concept by the occurrence of a macrocytic anemia in many subjects with advanced Laennec's cirrhosis;[12] however, the cause of this macrocytic anemia is obscure inasmuch as it usually does not respond to the administration of known maturation factors. More recently, evidence has been adduced suggesting that yet another mechanism is operative in the anemia of cirrhosis: namely, increased red cell destruction[13–16] resulting either from hypersplenism or immune mechanisms. Dameshek[17] and others[18,19] have reported typical instances of the hypersplenic state in patients with Laennec's cirrhosis; however, the response to splenectomy is not salutary in many instances[20] and the diagnosis of congestive splenomegaly or Banti's syndrome can only be made by inference in patients displaying pancytopenia and a normal bone marrow. Jandl[14] and Chaplin and Mollison,[13] employing the Ashby technic,[21] have shown that the life span of the red cells is decreased in patients with cirrhosis; however, it cannot be ascertained from the reports whether or not the patients had received blood transfusions prior to the studies, and it is possible that immune mechanisms incident to previous transfusion therapy may have contributed to the abbreviated life span encountered. Employing the radiochromium-tagged red cell technic, Sutherland[22] could demonstrate no decrease in the survival time of red blood cells in four cirrhotic subjects.

Despite low hemoglobin values and red cell counts, the twenty subjects comprising this report displayed no anemia in the sense of a decrease in red cell mass. On the other hand, previous studies utilizing isotope-labeled red cells have indicated an actual diminution in red cell mass in subjects with portal cirrhosis. Hyde et al.,[4] employing radiophosphorus-labeled red cells, found decreased values for red cell mass in 75 per cent of twenty-eight patients with cirrhosis. Chodos and his group,[23] using the same method, concluded that the anemia of cirrhosis represents an absolute reduction in red cell mass but also noted that expansion of the plasma volume may contribute to a relative reduction in hemoglobin concentration. According to this latter group, the total circulating hemoglobin was significantly decreased in a

majority of the patients and an increase toward normal accompanied clinical improvement. They also found that the hemoglobin concentration did not always reflect the level of total circulating hemoglobin. In the present study the mean red cell mass was 28.4 cc./kg. in twenty subjects with Laennec's cirrhosis who had neither bled nor required blood transfusions for a four-month period prior to the study; this slightly exceeded the mean red cell mass of 27.6 cc./kg. in the normal group. However, in eight of the patients the values for red cell mass were toward the lower limit of the normal range (20 cc./kg.). When the patients with polycythemia were excluded, the mean red cell mass was 25.5 cc./kg.; again, this did not differ significantly from the normal group. Following clinical improvement there was no significant change in red cell mass. There was, in fact, remarkable constancy of this function. The observation by Chodos[23] that hemoglobin concentration does not accurately reflect red cell mass is supported by the discrepancy between the hemoglobin and hematocrit values and the values for red cell mass herein reported. The mean hemoglobin concentration for these twenty patients was 11.6 gm. per cent and the mean packed cell volume was 38 per cent. Therefore, if a decrease in the circulating red cell mass was employed as the criterion, there was no anemia in the twenty subjects comprising this report. If the red cells were being destroyed at a rapid rate, increased red cell production must have effectively compensated for the loss from the circulating red cell mass. Although it must be concluded that anemia (resulting from blood loss, decreased red cell production or increased red cell destruction) may complicate the course of Laennec's cirrhosis, in many instances the low hemoglobin values represent an expansion of the plasma volume rather than an actual shrinkage of the red cell mass and hence are not indicative of true anemia.

Alterations in plasma volume incident to clinical improvement and the disappearance of ascitic fluid were of considerable magnitude in seven of the subjects; however, the plasma volume increased in four of the patients and decreased in three. The direction or degree of change could not be correlated with the presence or absence of esophageal varices, cyanosis, anemia or depression of the serum albumin. Measurements were made following paracentesis in only two subjects and in one patient, B. W., there was a distinct rise in plasma volume ninety minutes following the procedure.

The results of this study, then, indicate that in this group of patients with Laennec's cirrhosis of the liver the total blood volume, plasma volume and red cell mass were normal unless esophageal varices and/or cyanosis were present. There was a significant increase in plasma volume in patients with esophageal varices, and an expanded red cell mass was encountered in those subjects who were cyanotic. The red cell mass was normal in the twenty patients studied, despite the fact that the hemoglobin concentration was decreased in many instances; this suggests that the "anemia of liver disease" may often be due to hemodilution and that, under circumstances of an expanded plasma volume, determinations of hemoglobin concentration and packed cell volume do not accurately reflect the mass of red cells. The changes in plasma volume and hematocrit following clinical improvement were unpredictable and erratic, permitting no conclusions regarding compartmental exchanges of fluid during the disappearance of ascitic fluid which accompanied clinical improvement.

CONCLUSIONS

1. Blood volume, red cell mass and plasma volume have been determined in twenty patients with Laennec's cirrhosis of the liver by the radiochromium-labeled red cell technic.
2. There was a significant increase in plasma volume only in patients with esophageal varices and/or cyanosis; in the absence of these features all of the measured functions were normal.
3. Plasma volume and red cell mass were increased in four cirrhotic subjects with cyanosis.
4. There was no true anemia (defined as a true decrease in circulating red cell mass) nor was there a significant rise in red cell mass following clinical improvement.
5. There was no consistent change in plasma volume following the recession of ascites.

Acknowledgment: The author is indebted to Dr. Carleton B. Chapman and Dr. William F. Miller for the studies quantitating the pulmonary arteriovenous shunt in patient H. B.

ADDENDUM

Since this paper was submitted for publication, another patient has been studied prior to and following the establishment of a portacaval

shunt. In addition to esophageal varices this patient exhibited striking collateral vascular channels on the abdominal wall. Following the shunt procedure, all evidence of collateral circulation disappeared and there was a concomitant decrease in plasma volume:

	Blood Volume (cc.)	Red Cell Mass (cc.)	Plasma Volume (cc.)
Patient J. A. C.			
Before shunt	93	30	63
After portacaval shunt	70	31	39

REFERENCES

1. Perera, G. A. The plasma volume in Laennec's cirrhosis of the liver. *Ann. Int. Med.*, 24: 643, 1946.
2. Hiller, G. L., Huffman, E. R. and Levey, S. Studies in cirrhosis of the liver. I. Relationship between plasma volume, plasma protein concentrations and total circulating proteins. *J. Clin. Investigation*, 28: 322, 1949.
3. Bateman, J. C., Shorr, H. M. and Elgvin, T. Hypervolemic anemia in cirrhosis. *J. Clin. Investigation*, 28: 539, 1949.
4. Hyde, G. M., Berlin, N. I., Parsons, R. J., Lawrence, J. H. and Port, S. The blood volume in portal cirrhosis as determined by P^{32} labeled red blood cells. *J. Lab. & Clin. Med.*, 39: 347, 1952.
5. Sterling, K. and Gray, S. J. Determination of circulating red cell volume in man by radioactive chromium. *J. Clin. Investigation*, 29: 1614, 1950.
6. Sutherland, D. A., McCall, M. S., Groves, M. T. and Muirhead, E. E. The survival of human erythrocytes estimated by means of cells tagged with radioactive chromium: a study of the normal state. *J. Lab. & Clin. Med.*, 43: 717, 1954.
7. Chaplin, H., Jr., Mollison, P. L. and Vetter, H. The body/venous hematocrit ratio: its constancy over a wide hematocrit range. *J. Clin. Investigation*, 32: 1309, 1953.
8. Eisenberg, S. The effect of congestive heart failure on blood volume as determined by radioactive chromium tagged red cells. *Circulation*, 10: 902, 1954.
9. Keys, A. and Snell, A. M. The combination of oxygen and hemoglobin in patients with liver disease. *J. Clin. Investigation*, 16: 684, 1937.
10. Wilson, R. H., Ebert, R. V., Borden, C. W., Pearson, R. T., Johnson, R. S., Falk, A. and Dempsey, M. E. The determination of blood flow through nonventilated portions of the normal and diseased lung. *Am. Rev. Tuberc.*, 68: 177, 1953.
11. Berggren, S. M. The oxygen deficit of arterial blood. *Acta physiol. scandinav.* (suppl. 11), 4: 9, 1942.
12. Wintrobe, M. M. and Shumaker, H. S., Jr. Occurrence of macrocytic anemia in association with disorder of liver, together with consideration of relation of this anemia to pernicious anemia. *Bull. Johns Hopkins Hosp.*, 52: 387, 1933.
13. Chaplin, H. and Mollison, P. L. Red cell life-span in nephritis and in hepatic cirrhosis. *Clin. Sc.*, 12: 351, 1953.
14. Jandl, J. H. Studies on the mechanism of the anemia of liver disease. *J. Clin. Investigation*, 33: 946, 1954.
15. Hyman, G. A. and Southworth, H. Hemolytic anemia associated with liver disease. *Am. J. M. Sc.*, 221: 448, 1951.
16. Watson, C. J. Hemolytic jaundice and macrocytic hemolytic anemia: certain observations in a series of 35 cases. *Ann. Int. Med.*, 12: 1782, 1939.
17. Dameshek, W. and Welch, C. S. Hypersplenism and Surgery of the Spleen, 2nd ed., p. 19. New York, 1953. Grune & Stratton.
18. Durham, R. H. The results of splenectomy in Banti's syndrome. *Ann. Int. Med.*, 34: 1372, 1951.
19. Limarzi, L. R., Jones, R. M., Paul, J. T. and Poncher, H. G. Sternal marrow in Banti's syndrome and other splenomegalic states. *Am. J. Clin. Path.*, 13: 231, 1943.
20. Howells, L. The treatment of splenic anemia in Banti's syndrome. *Lancet*, 1: 1320, 1938.
21. Ashby, W. The determination of the life of the transfused blood corpuscles in man. *J. Exper. Med.*, 29: 267–281, 1919.
22. Sutherland, D. Personal communication.
23. Chodos, R. B., Denton, J., Ferguson, B. and Ross, J. F. The clinical significance of the blood volume in the anemia of portal cirrhosis. *Clin. Res. Proc.*, 1: 111, 1953.

Splenomegaly in Sickle Cell Anemia*

R. Janet Watson, M.D., Herbert C. Lichtman, M.D., *and* Henry D. Shapiro, M.D.

New York, New York *Paterson, New Jersey*

The spleen has long been of special interest in sickle cell anemia because of its variations in size, the extremes ranging from 1.5 gm.[1] to 1,850 gm.[2] It is the general consensus that the spleen tends to be enlarged more often in early childhood, and that it shrinks later in life, presumably as the result of repeated infarctions due to thrombosis of enmeshed sickled cells within its vascular bed. There are a few notable exceptions to these trends; spleens weighing less than 10 gm. have been reported in children,[3] and palpable spleens have been reported in adults.[4] The progressive changes in the size of the spleen[5] are caused by successive congestion of the pulp, hemorrhages surrounding the malpighian corpuscles, infarcts and intimal hyperplasia, and narrowing of the vessel lumina with the ultimate development of siderotic nodules, fibrosis and atrophy.

The relationship of the degree of anemia and the age of the patient to the size of the spleen, the possible occurrence of hypersplenism and the possible therapeutic value of splenectomy are all problems which require detailed and long term studies of individual patients—and these problems constitute the subject of this paper. This is an extension of previous work reported in 1952 in abstract form.[6]

With the discovery of abnormal hemoglobins other than sickle (S) hemoglobin, it has become important to verify the diagnosis of sickle cell anemia by the demonstration of the presence of homozygous S hemoglobin, now practical by use of filter paper electrophoresis. Both thalassemia-sickle cell disease and hemoglobin C-sickle cell disease are commonly associated with splenomegaly[7] and may masquerade as atypical instances of sickle cell anemia because of the positive sickling preparation.

MATERIAL AND METHODS

A total of 115 patients with sickle cell anemia seen at Kings County Hospital were studied in sufficient detail to warrant inclusion in this study. The majority were observed for more than four years, and some as long as seven years, both on the wards and in the Hematology Clinic. Basic studies included complete blood counts, the calculation of erythrocyte indices, enumeration of reticulocytes, measurement of icterus indices, performance of sickling tests, and the determination of hemoglobin type (homozygous S) using the method of filter paper electrophoresis described by Larson and Ranney.[8] A fresh solution of 1 per cent $NaHSO_3$ was used for sickling the erythrocytes, and the presence of long processes was ascertained. The stained blood smears were examined for the presence of target cells and "irreversible" sickle cells, which were seen in all cases. All of the patients had hemolytic anemia, usually normocytic and normochromic in type, with reticulocytosis and an elevated icterus index. Survival of red cells was studied in two patients by means of the Ashby technic.[9] Patients were considered to have splenomegaly when the spleen was easily palpated below the costal margin in quiet respiration by all observers. Because the study originated in the Department of Internal Medicine, there has probably been a tendency to see relatively more adults and to follow these more closely. One-half of the patients (58 of 115) were twenty or more years of age.

RESULTS

1. Incidence of Splenomegaly. Of 115 patients with sickle cell anemia, twenty-one, or 18 per cent, had splenomegaly. (Table I.) Fourteen patients, or two-thirds of those with enlarged spleens, were in the first decade of life, although only one-third of the entire series was in that age group. (Table II.) In contrast to the incidence of splenomegaly in patients of ten years and under, only eight of seventy-eight patients over the age of ten had splenomegaly, an incidence of 10 per cent. Ehrenpreiss and Schwinger[10] found roentgenographic evidence of splenomegaly in 25 per cent of the seventy-two patients they examined. Most of their patients had been studied by us.

In six children less than ten years of age the splenomegaly was transient. In this group the

* From the Departments of Pediatrics and Medicine, Kings County Hospital, State University of New York, College of Medicine, Brooklyn, New York. This work was supported in part by a grant from Lederle Laboratories, Pearl River, New York.

spleen edge became non-palpable in a period of six months to five and a half years after it was first felt. In one twenty-one year old adult transient enlargement, lasting one year, was noted. (Table III.) The degree of enlargement was relatively less in these patients, the spleen being no more than 5 cm. below the costal margin in any instance. If the patients in the younger age group had been followed more closely it is possible that transient splenomegaly would have been observed more often. Although the spleen is thought to be enlarged to about three times the normal size by the time it is palpable at the costal margin, it is more difficult to assess the significance of palpable spleens in very young children because it is said that they are occasionally palpable under normal conditions.

It will be seen in Table I that four of the patients with splenomegaly were not available for electrophoresis studies of their hemoglobin. Nevertheless, they were included in this series because of the high number of irreversible sickle cells in the blood smear (not seen in any other disease), the presence of long processes in the sickle cell preparation, and a typical clinical picture of severe crises. In one case the diagnosis was verified at autopsy.

TABLE I
SICKLE CELL ANEMIA IN TWENTY-ONE CASES WITH SPLENOMEGALY

Case No.	Age (yr.)	Spleen Size (cm. below costal margin)	Average Hemoglobin (gm. %)
1*	1	3†	9
2*	1	4†	7½
3	1½	5†	7
4*	2	12	9
5	2½	5†	8
6	3	4	7
7*	3	8	7
8	5	8	5½
9	5	2†	7½
10	8	4	8½
11	7	8	5½
12	8	1	9½
13	8	2†	8
14	10	15	5½
15	11	12	6½
16	16	8	9
17	21	5†	10
18	24	13	5
19	27	6	4½
20	34	1	8
21	36	2	12

* Not available for filter paper electrophoresis.
† Spleen subsequently became no longer palpable. (Age at the time of splenomegaly is given.)

TABLE II
INCIDENCE OF SPLENOMEGALY

Ages* (yr.)	With Splenomegaly		Without Splenomegaly		All Cases	
	No.	Hemoglobin (gm. %)	No.	Hemoglobin (gm. %)	No.	Per cent with Splenomegaly
1– 9	13	7½	24	8	37	35
10–19	3	7	17	8½	20	15
20–29	3	6½	43	8½	46	6.5
30–39	2	10	7	9	9	22
40–49	0	....	2	9½	2	0
50–59	0	....	1	9	1	0
Total	21	....	94	...	115	18.3

* The youngest patient was one year old; the oldest, fifty-four.

TABLE III
TRANSIENT SPLENOMEGALY IN SICKLE CELL ANEMIA

Case No.	Spleen First Palpable		Age (yr.) When Spleen Became Non-palpable
	Size (cm.)	Age (yr.)	
1	3	1	2
2	4	1	2
3	5	1½	7
5	5	2½	4
9	2	5	5½
13	2	8	9
17	5	21	22

2. Evidence of Hypersplenism. Leukopenia was observed in only one patient (Case 17) in the series. The white count of 4,000 per cu. mm. has persisted for three years although the spleen is no longer palpable. Whether the leukopenia has any relationship to the splenomegaly is questionable. Thrombocytopenia was not observed in any patient.

In order to determine whether or not splenomegaly was associated with an enhancement of

the anemia in sickle cell anemia, it was necessary to study the range of the hemoglobin in uncomplicated cases. We have been impressed with the fact that most patients with sickle cell anemia have very little if any fall in hemoglobin associated with their painful crises. Most of them have hemoglobin values varying from 7 to 9 gm. per cent when seen in the clinic during symptom-free periods. Crises necessitating hospitalization are usually characterized by fever, bone and joint pains, and abdominal pains without a further fall in hemoglobin. They are physiologically well adjusted to their anemia and symptoms attributable to it do not develop unless their hemoglobin falls to 6.5 gm. per cent or less, at which time weakness and considerable dyspnea on exertion usually develop. When this occurs administration of blood will abolish these symptoms but does not seem to have a beneficial effect upon the painful symptoms of the crises or shorten the attack. Hence it has been our custom to transfuse patients only if their hemoglobin was less than 6.5 gm. per cent, or the hematocrit less than 20 per cent. In the great majority of cases not more than one transfusion a year has been necessary.

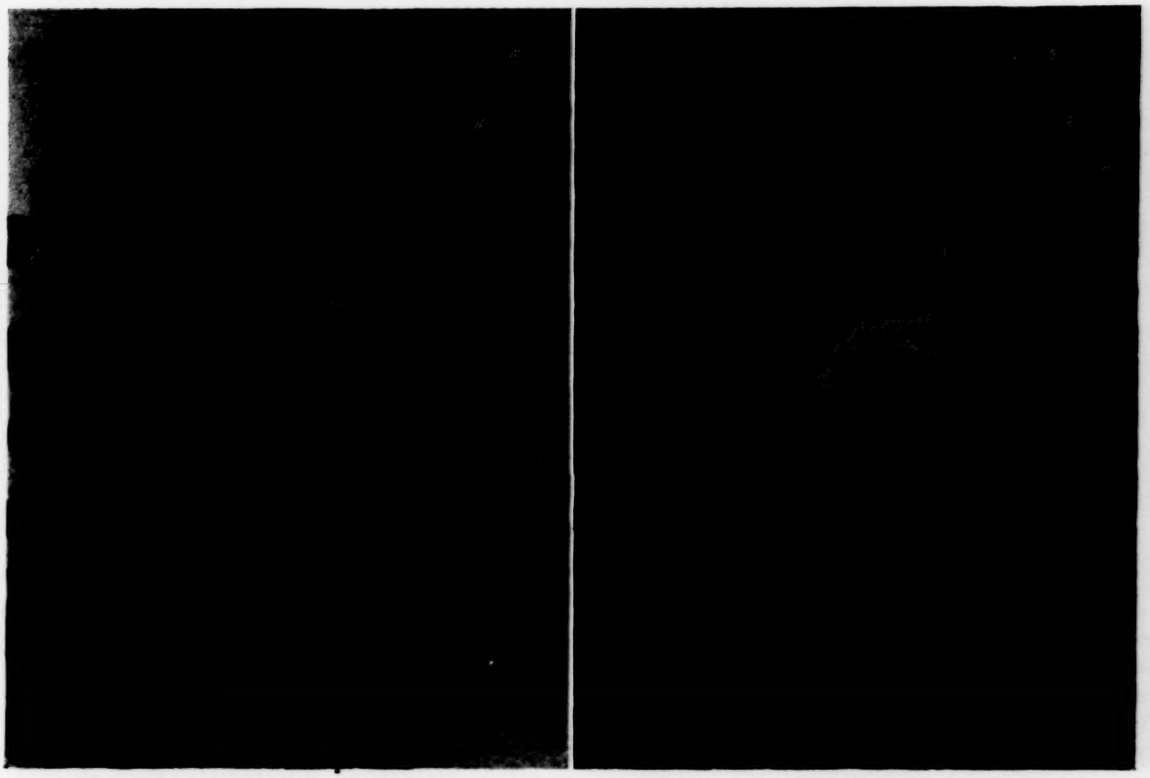
Unusually severe anemia with a high transfusion requirement has been encountered in six of our patients (Cases 8, 11, 14, 15, 18 and 19) who had a considerable degree of splenomegaly. (Table I.) Electrophoresis of the hemoglobin showed it to be homozygous S in all instances. The erythrocytes showed the increased resistance to hypotonic saline usually observed in sickle cell anemia. The Coombs test was negative in all patients. The range of reticulocytosis was quite variable, and was about the same as in most cases of sickle cell anemia—3 to 30 per cent—with the exception of Case 14, in which the reticulocytosis was as high as 70 per cent at one time. X-rays of the long bones and spine showed the characteristic changes of sickle cell anemia. Only one patient (Case 18) of the six had the "hair on end" finding in the x-ray of the skull. Diffuse calcification of the spleen was seen in the x-rays of Cases 18 and 19. All patients showed erythroid hyperplasia of the bone marrow.

In two patients (Cases 14 and 15) life-span studies of transfused normal red cells showed very short survival before splenectomy and normal survival afterwards. The removal of spleens weighing 1,300 and 760 gm., respectively, in these patients was followed by a rapid rise in hemoglobin, which has been sustained in the two years since operation so that neither patient has required blood transfusions. Splenectomy was performed in one patient (Case 8) only one month previous to writing; consequently it is too soon to evaluate the results. The spleen has not yet been removed in the other three patients (Cases 11, 18 and 19). In an attempt to understand the pathogenesis of the anemia in these patients it is necessary that their case records be given in detail.

CASE REPORTS

CASE 14. This patient, a thirteen year old Negro girl, had bouts of pain in the joints, arms and legs about once a year from the age of three to seven and a half years. She was then seen by us for the first time and the spleen was noted to be enlarged to 7 cm. below the costal margin. The hemoglobin was 8 gm. per cent. A year later the hemoglobin was 5.5 gm. per cent, and the spleen edge had extended to the umbilicus. The following year she was admitted to the hospital with a hemoglobin of 4.5 gm. per cent and enlargement of the spleen to the iliac crest. In fact, in the years between the ages of seven and a half and ten and a half the hemoglobin averaged only 5.5 gm. per cent, with one value as low as 3.5 gm. per cent, despite transfusions of 500 cc. of blood given monthly because of dyspnea, fatigue and weakness. It is interesting to note that during this time she had no painful crises. The Coombs test was negative. In April, 1952, a spleen weighing 1,300 gm. was removed. Microscopic sections of the spleen showed considerable hemosiderosis and engorgement of the sinusoids with sickled cells. No erythrophagocytosis or infarcted areas were seen. Blood studies made at the time of operation are summarized in Table IV.

Specimens of blood were collected simultaneously from the femoral artery, splenic vein and splenic pulp, and were examined for the number of cells circulating in the sickled state, both in the reversible and in the irreversible forms. Cells circulating in the reversible sickled shapes were counted, using Sherman's method[11] of collecting blood under oil and fixing it in 10 per cent formalin in physiologic saline solution. In sickle cell anemia usually about 20 to 60 per cent of the erythrocytes in venous blood are found to have sickled processes, which resemble those seen in bisulfite preparations. These cells presumably revert to the biconcave disc upon exposure to air since they are never seen in the stained blood smear. The "irreversible" sickle cells seen in the peripheral blood film are dense, elongated forms which lack multiple sickle cell processes. These cells can also be identified in the wet sealed chamber and do not revert to the biconcave shape upon exposure to an atmosphere of 100 per cent oxygen (although they will develop multiple sickle processes if exposed to 100 per cent CO_2 or



1 2

FIG. 1. Case 14. Peripheral blood smear. Many target cells and one irreversible sickle cell.

FIG. 2. Case 14. Contact smear of spleen. No target cells and many irreversible sickle cells.

N_2 gas).[12] Presumably they also are unable to resume their normal shape *in vivo*.

In this patient it is evident that the number of reversibly sickled cells was much higher in the splenic vein and pulp than in the femoral artery, as would be expected because of the lower oxygen tension in venous blood. The number of irreversibly sickled cells, as seen in smears stained with Wright's stain, was much higher in the splenic pulp than in the splenic vein, femoral artery and peripheral blood. (Figs. 1 and 2.) The fact that there were fewer reticulocytes in the splenic pulp can probably be ascribed to the relatively large number of irreversibly sickled cells (which occur rarely in reticulocytes[12]) in the splenic pulp. The patient's reticulocytes varied from 20 per cent to as high as 70 per cent before operation. Blood was drawn from the patient at a time when her reticulocytes were 67 per cent, and was then given to a healthy eleven month old child whose red cells did not sickle. The transfused reticulocytes disappeared within four days. (Fig. 3.) Incubation of the patient's blood *in vitro* at 37°C. gave similar results.

TABLE IV
RESULTS OF BLOOD STUDIES MADE AT SPLENECTOMY* (CASE 14)

Blood Studies	Femoral Artery	Splenic Vein	Splenic Pulp
Red blood cells sickled by $NaHSO_3$(%)	69.0†	72.5†	74.0†
Reversibly sickled red blood cells (%)	16.0	27.5	32.0
Irreversibly sickled red blood cells (%)	1.0	2.5	18.0
Reticulocytes (%)	19.0	16.5	9.5
Irreversibly sickled reticulocytes (%)	0.05	0.0	0.0
Hemoglobin (gm. %)	5.4	5.3	5.8

* Spleen weighed 1,300 gm.

† This figure is not 100 per cent because the patient had been previously transfused with normal erythrocytes.

The survival of the patient's cells in the normal recipient was determined both by the Ashby technic and by counting sickle cells.[13] (Fig. 3.) It is evident that the results by both technics are comparable, and that although the total life span of the sickle cells was about thirty days, half of the cells were destroyed in six days. A curve showing two components of cells in sickle cell disease has been demonstrated by others.[14,15] Since the survival of the reticulocytes was only four days, it does not seem likely that their selective de-

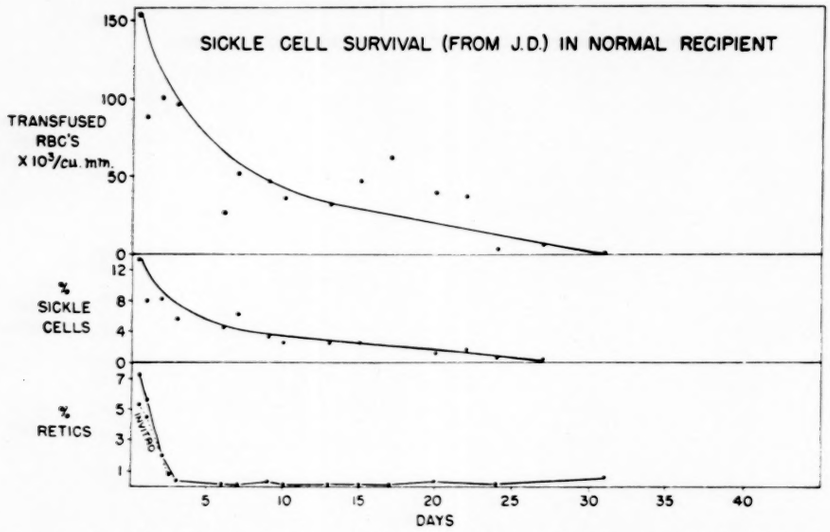


FIG. 3. Case 14. Survival curves of the patient's erythrocytes and reticulocytes in a normal recipient.

struction could account for the rapid destruction of the sickle cells. It is more likely that reticulocytes matured within four days and were destroyed after they had become non-reticulated and sickled erythrocytes.

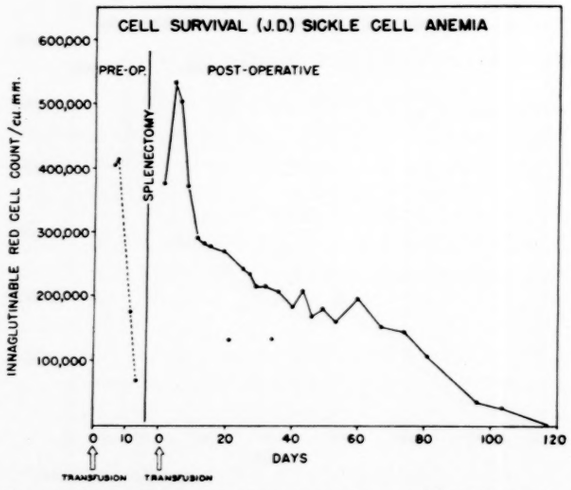


FIG. 4. Case 14. Survival curves of normal erythrocytes in the patient before and after removal of an enlarged spleen weighing 1,300 gm.

Life-span studies of normal blood transfused into the patient before and after splenectomy are illustrated in Figure 4, and show that the short survival of fourteen days was lengthened to a normal survival of 120 days after removal of the spleen. This indicates that an extracorpuscular type of defect was present in addition to the expected intracorpuscular defect.

The patient's hematologic improvement after splenectomy is given in Table v. In the two and a half years since operation the hemoglobin has varied from 7 to 9 gm. per cent, with an average of 8 gm. per cent. The patient has been very well except for three episodes of pain in the legs and fever, requiring hospitalization for less than a week each time. However, nine months after the operation she was admitted because of a spiking temperature to 104°F.

TABLE V
HEMATOLOGIC IMPROVEMENT AFTER SPLENECTOMY FOR HYPERSPLENISM (CASE 14)

Hematology	Pre-operatively	Five Days Post-operatively	Two Years Post-operatively
Red blood count (million per cu. mm.)	1.84	3.47	3.20
Hemoglobin (gm. %)	5.3	10.7	8.6
Hematocrit (%)	19	33.5	28
Mean corpuscular volume (cu. micra)	103	102	87.5
Mean corpuscular hemoglobin concentration (%)	28	30	27
Mean corpuscular hemoglobin (micromicrogm)	29	30	31
Reticulocytes (%)	67.2	1.0	1.9
Normoblasts/100 white blood cells	70	0	0
White blood count/cu. mm.	13,100	11,500	11,600
Icterus index (units)	15	10	5
Transfusion requirement	500 cc. every 3 to 4 wk.	0	0

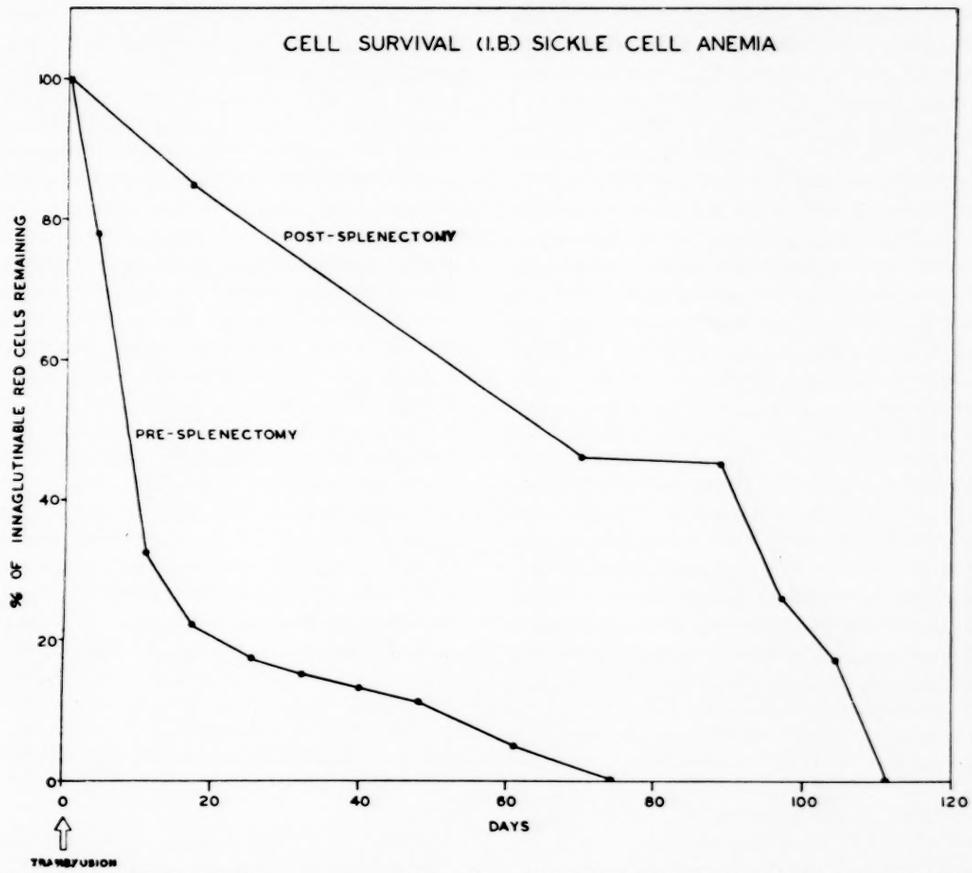


FIG. 5. Case 15. Survival curves of normal erythrocytes in the patient before and after removal of an enlarged spleen weighing 750 gm.

associated with shaking chills. A heavy infestation of red cells by quartan malaria was found in the blood film. The patient responded well to chloroquine and has had no relapses. Review of previous blood films and spleen slides failed to show the presence of malarial parasites or pigment. (Allison has noted protection against the subtertian form of malaria in sicklemia.[16]) The patient has required no transfusions since splenectomy.

CASE 15. The patient, a thirteen and one-half year old Negro girl, was found to be anemic at the age of three years, at which time the diagnosis of sickle cell anemia was made. Between the ages of six to eleven years she was transfused every two to three months because of weakness and fatigue. She had never had painful crises. We first saw the patient when she was eleven years old. At this time the spleen was found to be 12 cm. below the costal margin. The hemoglobin was 5.5 gm. per cent, red blood count 1.7 million per cu. mm., hematocrit 15 per cent, reticulocytes 18.6 per cent and the icterus index 15 units. The Coombs test was negative. Half of the transfused normal red cells in this patient survived only fourteen days (Fig. 5), thus substantiating the clinical impression that in addition to the sickle cell anemia there was a superimposed factor of hypersplenism. Splenectomy was therefore performed and a large spleen, weighing 750 gm., was removed. Splenic contact smears showed 32 per cent irreversible sickle cells, whereas the peripheral blood showed only 0.5 per cent. Microscopic sections showed the presence of considerable hemosiderosis in addition to marked congestion of the splenic pulp with sickled cells. No infarction was present. The Ashby test was repeated after the operation and normal cells were then found to have a normal survival time within the patient. (Fig. 5.) During the two and a half years since the operation the patient's hemoglobin has been maintained at 9.5 to 11 gm. per cent, with an average of 10 gm. per cent, without the necessity of any transfusions. The reticulocytes have varied from 3.2 to 22.6 per cent. The patient has been entirely free of symptoms and has been able to attend school regularly.

CASE 8.* This patient, a five year old Negro girl, was first diagnosed as having sickle cell anemia at the

* We are indebted to Dr. Felix Feldman for his courtesy in letting us see this patient and for supplying us with some of the data.

age of twenty months, at which time she was admitted to St. John's Hospital with a hemoglobin of 2.1 gm. per cent and a spleen which was felt 2 cm. below the costal margin. The only time she ever had a painful crisis was six months later when she was admitted to the hospital because of abdominal pain. The hemoglobin then was 9.4 gm. per cent. In the following two and a half years her only symptoms have been weakness and anorexia associated with a severe anemia, the hemoglobin varying between 3.6 and 6.7 gm. per cent, with an average of 5.5 gm. per cent. She has received twenty-seven transfusions, totalling 7,200 cc., or an average of 220 cc. of blood a month. During this time the spleen has gradually enlarged; when she was first seen by us on October 28, 1954, it was felt 8 cm. below the costal margin. At that time (three days after a blood transfusion) the hemoglobin was 6.3 gm. per cent, red blood count 1.97 million per cu. mm., hematocrit 23.5 per cent, reticulocytes 23.4 per cent and the icterus index 75 units. On November 23rd splenectomy was performed. The spleen weighed 520 gm., and microscopic sections showed marked congestion of the pulp with sickled cells and considerable hemosiderosis but no areas of infarction. On discharge twelve days postoperatively the hemoglobin was 11.2 gm. per cent and the red blood count 3.8 million per cu. mm. It is too soon to evaluate the effect of splenectomy but it is hoped that her transfusion requirement will be markedly reduced, as has occurred in Cases 14 and 15. On January 19, 1955, the hemoglobin was 8.8 gm. per cent and the red blood count 3.08 million per cu. mm. In December, 1955, her clinical and hematologic improvement had been sustained without the need of blood transfusions.

Case 11. This patient, an eight year old Negro girl, was first diagnosed as having sickle cell disease at the age of two years when she was admitted to another hospital for convulsions and pains in the legs and abdomen. In the past six years she has had attacks of abdominal pain and pain in the legs, anorexia and fatigue occurring every two to four months. She was first seen by us in 1951 at which time the hemoglobin was 3 gm. per cent and the spleen was not palpable. On January 26, 1953, the spleen first became palpable 2 cm. below the costal margin, gradually enlarged to a maximum of 8 cm. on August 23, 1953, and has remained about that size to the present time. In the past three years the hemoglobin has varied from 3.0 to 6.2 gm. per cent, with an average of 5½ gm. per cent; the patient has received a transfusion about every three months. When she was last seen (one month after a transfusion) the hemoglobin was 6.2 gm. per cent, red blood count 2.46 million per cu. mm., hematocrit 20 per cent, reticulocytes 6.0 per cent and the icterus index 20 units. Because this patient's anemia is more severe than that of the average patient with sickle cell anemia, and because of the enlarging spleen, splenectomy is planned.

Case 18. The patient, a twenty-six year old Negro man, was first diagnosed as having sickle cell anemia at the age of seven years, at which time he had an attack of generalized joint pains and fever and was found to have splenomegaly and anemia, for which he received a transfusion. Since then he has continued to have splenomegaly and has had attacks of joint pains, usually in the knees, back and elbows, every three weeks, and pains in the splenic area twice yearly, but received no further transfusions until 1953. His pains have been limited to the joints and have never involved the long bones. In 1953 an ulcer on the left ankle developed, which healed slowly over a period of three months. He was first seen by us on July 9, 1953, at which time the hemoglobin was 4.5 gm. per cent and the spleen was 10 cm. below the costal margin. Since then the spleen has enlarged to 13 cm. and the hemoglobin has averaged 5 gm. per cent. During this time he has received twelve pints of blood, or an average 500 cc. every six weeks. He has continued to have attacks of pain in the joints and in the left upper quadrant of the same frequency and severity, but has had a great increase in weakness and dyspnea on exertion. At his last admission on November 28, 1954 hemoglobin was 5.5 gm. per cent, red blood count 1.8 million per cu. mm., hematocrit 21 per cent, reticulocytes 17.0 per cent and icterus index 50 units. Splenectomy is planned because of his increasing transfusion requirement and enlarging spleen.

Case 19. This patient, a twenty-nine year old Negro woman, had frequent bouts of pain in the bones, joints and abdomen since early childhood up until 1951. During that period of time the hemoglobin averaged about 8 gm. per cent and she received about two transfusions annually. In 1952 the spleen became palpable for the first time, and extended 6 cm. below the costal margin. This splenomegaly has persisted to the present time. Since 1951 the hemoglobin has averaged only 4.5 gm. per cent, often being as low as 3 gm. per cent, despite monthly transfusions of 500 to 1,000 cc. of blood. She has had no more painful crises but has suffered from severe weakness, dyspnea and dizziness. Recently ankle edema and an enlarging tender liver developed, for which she was digitalized. She is our only patient with sickle cell anemia who has had serious heart failure; we assume that this is due to myocardial anoxia caused by the unusually severe chronic anemia. This patient has refused splenectomy.

3. Study of Irreversibly Sickled Cells in the Spleen. The knowledge that irreversibly sickled cells can be produced artificially under conditions of stasis and anoxemia[17] led to the investigation of their relative numbers in the spleen, a good organ for the study of the effect of stasis.[18] Splenic aspirations were performed in eight patients with splenomegaly, and the number of

irreversible sickle cells per 1,000 red cells in the peripheral blood was compared with that in the splenic material. It was found that ratios varied from 1:2 to 1:14. (Table VI.) Contact smears were made of the spleen at operation in Cases 14 and 15, and ratios of 1:18 and 1:64,

TABLE VI
SPLENIC ASPIRATION IN EIGHT CASES OF SICKLE CELL ANEMIA

Case No.	I.S.*/1,000 Red Blood Cells	I.S./1,000 Red Blood Cells	Ratio I.S. Blood/Spleen
	Blood	Spleen	
1	70	220	1:3
7	5	68	1:14
9	42	378	1:9
14	21	77	1:4
15	6	45	1:7
16	125	244	1:2
17	2	6	1:3
19	2	18	1:9

* I.S. = irreversible sickle cells.

respectively, were obtained. Comparably fewer irreversible sickle cells were obtained when splenic aspiration was performed. Ratios of 1:4 and 1:9 were obtained. This is probably due in part to the inevitable dilution incident to aspiration and in part to the possibility that enmeshment of the sickled cells in the spleen may very well tend to prevent their aspiration. Studies in Case 14 at the time of operation are shown in detail in Table IV. Study of the smears of splenic material failed to reveal the presence of any target cells, in contrast to their abundance in the peripheral blood smears. (Figs. 1 and 2.) Erythrophagocytosis was not seen in any of the splenic slides.

COMMENTS

Incidence of Splenomegaly in Sickle Cell Anemia. Splenomegaly has long been known to be an occasional feature of sickle cell anemia,[3] especially in early childhood, although precise detail is lacking. Scott and co-workers[19] noted splenomegaly in eighty-four of 151 hospital admissions of thirty-seven patients with sickle cell anemia under the age of twelve years but did not state whether the patients with splenomegaly had more or less frequent admissions than the others. Anderson and Ware[20] reported splenomegaly in fourteen of twenty-two cases in the first decade of life. Grover[21] reported palpable spleens in nineteen of forty-seven patients of all ages but the age distribution of those with splenomegaly was not given. Henderson[4] found enlarged spleens in only three adults of a total of fifty-four cases. Conley and Smith[7] state that they have never seen splenomegaly in patients with sickle cell anemia who were over the age of ten years. In our series of 115 patients fourteen of the twenty-one with splenomegaly were less than ten years of age. (Tables I and II.) The incidence of splenomegaly was 33 per cent in the first decade of life and 10 per cent thereafter. Therefore, splenomegaly is not uncommon in sickle cell anemia but seems to be much more frequent in sickle cell (S)-hemoglobin C disease and in thalassemia-sickle disease, being reported in well over half of the cases.[22] Our experience with sickle cell (S)-hemoglobin C disease differs from that of others in that only two of ten patients had splenomegaly and they were both children.[23]

Factors Affecting Size of Spleen in Sickle Cell Anemia. In seven patients (six children and one adult) splenic enlargement was transient (Table III) and probably represented the successive operation of two different pathologic processes. It has been assumed that the shrinkage of the spleen is due to ischemic infarction as a result of sickling and thrombosis occurring within the sinusoids.[5,24] Why this particular organ is selectively involved in the process of infarction may be related to the special anatomic features of the spleen. These seem to encourage stasis, and hence anoxia, as has been emphasized by Ham and Castle.[18]

Shen et al.[17] were able to produce irreversibly sickled red cells *in vitro* under experimental conditions which simulated stasis, by incubation of sickle cells in an atmosphere of a mixture of 10 per cent carbon dioxide and 90 per cent nitrogen gas. An "irreversible" sickle cell is an elongated dense red cell which, as the name implies, cannot revert to the biconcave disc shape upon exposure to oxygen and hence can be seen in the ordinary stained blood film of patients with sickle cell anemia. (Fig. 1.) These cells, however, are able to produce the filamentous processes when exposed to low oxygen tension.[12] It is not known whether irreversible sickle cells differ in their viscosity and fragility from cells in the reversible sickled form. Al-

though their numbers fluctuate considerably from time to time, neither we nor others have been able to correlate their number with the presence or absence of crises or with the severity of the anemia. It is interesting to note the relatively high proportion of the irreversible sickle cells seen by us in the contact smears of the spleen at operation in Cases 14 (Fig. 1 and 2) and 15 and in the splenic aspirations, as shown in Table VI. It seems likely that these cells are formed in the splenic pulp and then trapped there because of their peculiar shape. Weisman and others[29] showed a relative increase in the number of sickle cells in spleens along with a marked increase of osmotic fragility of these cells. This is analogous to but less marked than the sequestration of spherocytes in congenital hemolytic anemia. Tomlinson[30] has shown in perfusion experiments that sickle cells are trapped in the pulp cords. The complete absence of target cells (thin cells) in the splenic smears of our patients probably is a reflection of the swelling of these cells in the spleen, with a relative increase in osmotic fragility.

Some twenty-one cases of splenic infarction associated with high altitude flying and sicklemia have been reported.[25–27] A similar case due to sickle cell disease has been described by Doenges et al.[28] who also emphasized the importance of establishing the exact diagnosis by electrophoresis of hemoglobin, a procedure which was not carried out by the preceding authors. Since sickle cells are known to assume their abnormal shape when oxygen tension is sufficiently lowered, the mechanism of splenic infarction in the high altitude fliers would seem obvious. However, none of the patients had symptoms relative to sickling in other parts of the body, such as pain in the abdomen, bones or joints, nor did they have evidence of severe hemolysis.

It may be, then, that under the unique conditions of stasis in the spleen, falling oxygen tension favors sickling[17] and the production of irreversible sickle forms. If these sufficiently obstruct the circulation in the spleen, ischemic infarction would result with the later development of atrophy and siderofibrosis.[5] The increased viscosity of cells in the sickled shape[18] increases the tendency to thrombosis. If the splenic circulation is not completely blockaded, increasing formation and sequestration of sickled cells, with enlargement of the spleen, would occur. Furthermore, cells in the sickled shape have a very high mechanical fragility[31,32] so that one could expect an increasing degree of hemolysis with increasing size of the spleen. The relatively small number of irreversible sickle cells in the circulating blood and the irregular fluctuation in their number may be due to the fact that irreversible sickle cells are destroyed within the spleen (as well as being formed there), and that their unique shape does not allow them ready access into the blood stream from the splenic pulp.

Effect of Splenomegaly on the Anemia in Sickle Cell Disease. In the seven cases of splenomegaly in which the enlargement was transient there was no unusual degree of anemia. However, six (Cases 8, 11, 14, 15, 18 and 19) of the remaining fourteen had an unusually severe anemia associated with an unusual degree of enlargement of the spleen. There seemed to be a rough correlation between the degree of enlargement of the spleen and the severity of the anemia. (Table I.) The negative Coombs test in all cases gave no evidence of an autoimmune mechanism. It would be necessary to do life-span studies of the patient's own erythrocytes within himself in order to find out whether the patient's enlarged spleen was an overactive graveyard for his own sickle cells. Unfortunately, it was not possible to do such studies in these patients. However, by means of the Ashby technic it was possible to demonstrate that survival of normal transfused cells was short before splenectomy and normal after splenectomy in two of our patients. (Figs. 4 and 5.) Since normal cells usually have a normal survival time in patients with sickle cell anemia,[14,15] this means that there was a superimposed extracorpuscular defect in these cases. Furthermore, the fact that both patients have maintained relatively good hemoglobin levels during the two years since splenectomy indicates that removal of the spleen removed an extracorpuscular factor that was injurious to the patient's own red cells or to the rate of erythropoiesis, as well as to the normal transfused cells. (These patients resemble certain cases of Cooley's anemia associated with hypersplenism.)[33]

Shotten et al.[2] have reported a similar patient, aged five years, in whom removal of a spleen weighing 1,850 gm. resulted in marked improvement during an eighteen-month period of subsequent observation. They reviewed twenty-three cases of splenectomy in sickle cell anemia reported in the literature. "Good" or "fair" results were obtained in nine of ten pa-

tients in whom the spleen extended to the iliac crest but in only one of five patients with smaller spleens. All but two of the patients were ten years old or younger.

Degree of Anemia in Sickle Cell Disease in the Absence of Splenomegaly. We have been impressed with the fact that most of our patients with sickle cell anemia maintain a relatively stable hematologic equilibrium between the forces of red cell formation and destruction, maintaining a hemoglobin that varies from 7 to 9 gm. per cent. (Table II.) The great majority of hospital admissions are the result of crises characterized by fever and pain in bones, joints and abdomen, without exacerbation of the anemia. However, four of our six patients (Cases 8, 14, 15 and 19) who had an unusually severe anemia, with hemoglobin values averaging from 4 to 6 gm. per cent, had very few or no painful symptoms. This may be due to the fact that at this low level of circulating red cells the viscosity of the blood, and therefore the tendency to thrombosis, is diminished. The patient in Case 19 had painful crises all her life until her anemia became severe in the last three years, during which time her only symptoms have been weakness and fatigue. The reverse of this was seen when we treated two patients with cobaltous chloride, producing a rise in hemoglobin from 7 to 11 gm. per cent.[34] This rise in hemoglobin was accompanied by such an increase in frequency and severity of bone and abdominal pain that the medication had to be discontinued. Although it is possible that cobalt had some other unknown deleterious effect on sickle cell anemia, it seemed likely that the exacerbation of symptoms was due to an increased incidence of thrombosis related to the increase in circulating sickle cells.

Splenectomy in Case 15 has resulted in maintenance of a relatively high hemoglobin concentration (10 gm. per cent) for over two years, yet this patient has had no sickle crises. This may be due to the fact that the spleen was the main site of sickling of the red cells. Since crises are known to occur in other patients with small spleens, it is probable that stasis and pooling of sickled cells takes place to a varying degree in the capillary bed of organs throughout the body. If these organs were accessible to the study of *in vivo* sickling, much could be learned about the dynamics of a crisis.

The Role of the Spleen in Sickle Cell Anemia. This may be postulated as follows: Circulating red cells become sickled in the spleen under the conditions of anoxia and stasis, and become irreversibly sickled if they remain sequestered for a long time. The increased mechanical fragility of the sickled cells results in hemolysis. In most instances the sickled red cells (with a high viscosity) within the spleen will obstruct the circulation in the capillaries causing ischemic infarction and subsequent fibrosis and shrinkage of the organ. However, if the sickle blockade is not complete the spleen will progressively enlarge, with increasing sequestration and destruction of red cells leading to exacerbation of the pre-existing anemia if bone marrow production is already maximal. With the advent of severe anemia, painful crises tend to diminish because there is less opportunity for circulating sickle cells to enmesh and obstruct the capillary flow of blood. Splenectomy in such cases will diminish the severity of the anemia. It should be performed if the spleen is considerably enlarged and if the anemia has become so profound that the patient is incapacitated by weakness and dyspnea and is further inconvenienced by the increasing necessity for blood transfusions. This vicious cycle is not a constant feature in patients with splenomegaly but was thought to occur in six of our twenty-one patients who had splenomegaly.

SUMMARY AND CONCLUSIONS

1. Palpable spleens were found in 18 per cent of 115 patients with sickle cell anemia, one to fifty-four years of age. Splenomegaly was present in 33 per cent of patients in the first decade of life and in 10 per cent of those older.

2. In seven patients the splenomegaly was transient. Six of these were children.

3. In six patients with a considerable degree of splenic enlargement the anemia was unusually severe, although painful crises tended to be less frequent. The existence of an extracorpuscular factor in blood destruction was demonstrated in two patients by the finding of a shortened survival of normal transfused erythrocytes before splenectomy and normal survival afterwards. The removal of the spleen resulted in marked clinical improvement and in a rise of hemoglobin so that further transfusions were not necessary.

4. A relatively high percentage of irreversible sickle cells was found in the splenic aspiration compared to that in the peripheral blood in eight patients with splenomegaly. This was

thought to indicate probable formation and sequestration of these cells in the spleen.

5. The mechanism and role of the spleen in sickle cell anemia is discussed.

ADDENDUM

Since the preparation of this paper, Smith and Conley (*Bull. Johns Hopkins Hosp.*, 96: 35, 1955) have reported eleven cases of splenic infarction at high altitudes in carriers of sickle cell trait verified by electrophoresis.

Acknowledgments: We are indebted to Dr. Lawrence Deutsch for the construction of our filter paper electrophoresis equipment, to Mrs. Ellen Eschenbrenner for the electrophoretic determinations, to Miss Jean Robinson for the Ashby determinations, and to Mr. Vernon Delgado and Miss Mildred Fishman for the hematologic work.

REFERENCES

1. Bridgers, W. H. Cerebral vascular disease accompanying sickle cell anemia. *Am. J. Path.*, 15: 353–361, 1939.
2. Shotten, D., Crockett, C. L. and Leavell, B. S. Splenectomy in sickle cell anemia: report of a case and review of the literature. *Blood*, 6: 365–371, 1951.
3. Margolies, M. P. Sickle cell anemia. *Medicine*, 30: 357–443, 1951.
4. Henderson, A. B. Sickle cell anemia. Clinical study of 54 cases. *Am. J. Med.*, 9: 757–765, 1950.
5. Diggs, L. W. Siderofibrosis of the spleen in sickle cell anemia. *J. A. M. A.*, 104: 538–541, 1935.
6. Lichtman, H. C., Shapiro, H., Ginsberg, V. and Watson, J. Splenic hyperfunction in sickle cell anemia, (Abst.). *Am. J. Med.*, 14: 516, 1952.
7. Conley, L. C. and Smith, E. W. Clinical features of genetic variants of sickle cell disease. *Tr. A. Am. Physicians*, 67: 261–267, 1954.
8. Larson, D. L. and Ranney, H. M. Filter paper electrophoresis of human hemoglobin. *J. Clin. Investigation*, 32: 1070–1076, 1953.
9. DeGowin, E. L., Sheets, R. F. and Hamilton, H. E. Studies with inagglutinable erythrocyte counts. I. A method for measurement of net gain or deficit of red cells in the human subject. *J. Clin. Investigation*, 29: 693–713, 1950.
10. Ehrenpreis, B. and Schwinger, H. N. Sickle cell anemia. *Am. J. Roentgenol.*, 68: 28–36, 1952.
11. Sherman, I. J. The sickling phenomenon with special reference to the differentiation of sickle cell anemia from the sickle cell trait. *Bull. Johns Hopkins Hosp.*, 67: 309–324, 1940.
12. Watson, J. A study of sickling of young erythrocytes in sickle cell anemia. *Blood*, 3: 465–469, 1948.
13. Altman, A. The survival of transfused erythrocytes in sickle cell anemia. *Tr. Roy. Soc. Trop. Med. & Hyg.*, 40: 901–904, 1947.
14. Singer, K., Robin, S., King, J. C. and Jefferson, R. N. The life span of the sickle cell and the pathogenesis of sickle cell anemia. *J. Lab. & Clin. Med.*, 33: 975–984, 1948.
15. Callender, S. T., Nickel, J. F., Moore, C. V. and Powell, E. O. Sickle cell disease: studied by measuring the survival of transfused red blood cells. *J. Lab. & Clin. Med.*, 34: 90–104, 1949.
16. Allison, A. C. Protection afforded by sickle-cell trait against subtertian malarial infection. *Brit. Med. J.*, 1: 290–294, 1954.
17. Shen, S. C., Fleming, E. M. and Castle, W. B. Studies on the destruction of red blood cells. V. Irreversibly sickled erythrocytes: their experimental production *in vitro*. *Blood*, 4: 498–504, 1949.
18. Ham, T. H. and Castle, W. B. Relation of increased hypotonic fragility and of erythrostasis to the mechanism of hemolysis in certain anemias. *Tr. A. Am. Physicians*, 55: 127–132, 1940.
19. Scott, R. B., Banks, L. O., Jenkins, M. E. and Crawford, R. P. Studies in sickle-cell anemia. II. Clinical manifestations of sickle-cell anemia in children. *J. Pediat.*, 39: 460–471, 1951.
20. Anderson, W. W. and Ware, R. L. Sickle cell anemia. *Am. J. Dis. Child.*, 44: 1055–1070, 1932.
21. Grover, V. The clinical manifestations of sickle cell anemia. *Ann. Int. Med.*, 26: 843–851, 1947.
22. Smith, E. W. and Conley, C. L. Clinical features of the genetic variants of sickle cell disease. *Bull. Johns Hopkins Hosp.*, 94: 289–318, 1954.
23. Unpublished data.
24. Kimmelstiel, P. Vascular occlusion and ischemic infarction in sickle cell disease. *Am. J. M. Sc.*, 216: 11–19, 1948.
25. Sullivan, B. H. Danger of airplane flight to persons with sicklemia. *Ann. Int. Med.*, 32: 338–342, 1950.
26. Cooley, J. C., Peterson, W. L., Engel, C. E. and Jernigan, J. P. Clinical trial of massive splenic infarction, sicklemia trait, and high altitude flying. *J. A. M. A.*, 154: 111–113, 1954.
27. Conn, H. O. Sickle-cell trait and splenic infarction associated with high-altitude flying. *New England J. Med.*, 251: 417–420, 1954.
28. Doenges, J. P., Smith, E. W., Wise, S. P. and Breitenbucher, R. B. Splenic infarction following air travel and associated with the sickling phenomenon. *J. A. M. A.*, 156: 955–957, 1954.
29. Weisman, R., Jr., Hurley, T. H., Harris, J. W. and Ham, T. H. Studies of the function of the spleen in the hemolysis of red cells in hereditary spherocytosis and sickle cell disorders. (Abst.). *J. Lab. & Clin. Med.*, 42: 965–966, 1953.
30. Tomlinson, W. J. Studies of the circulation of the spleen in sicklemia and sickle cell anemia. *Am. J. Path.*, 21: 877–887, 1945.
31. Shen, S. C., Castle, W. B. and Fleming, E. M. Experimental and clinical observation on increased mechanical fragility of erythrocytes. *Science*, 100: 387–389, 1944.
32. Lange, R. D., Minich, V. and Moore, C. V. Effect of oxygen tension and of pH on the sickling and mechanical fragility of erythrocytes from patients with sickle cell anemia and the sickle cell train. *J. Lab. & Clin. Med.*, 37: 789–802, 1951.
33. Lichtman, H. C., Watson, R. J., Feldman, F., Ginsberg, V. and Robinson, J. Studies on thalassemia. *J. Clin. Investigation*, 32: 1229–1233, 1953.
34. Unpublished data.

Survival of Diabetic Patients after Myocardial Infarction*

R. F. BRADLEY, M.D. *and* J. W. BRYFOGLE, M.D.†

Boston, Massachusetts *Philadelphia, Pennsylvania*

CORONARY artery disease is now the leading cause of death in diabetics. Among the last 656 deaths (1950–1952) recorded by the Joslin Clinic 47.6 per cent were due chiefly to the effects of coronary sclerosis upon the heart.[1] Since mortality from coronary artery disease has also increased to 22.7 per cent in the total population, it seems reasonable to speculate that diabetes has further accelerated and increased the extent of these atherosclerotic changes.[2,3]

INCIDENCE

Non-diabetic. The true incidence of coronary artery disease in the non-diabetic population is unknown but some idea of its great prevalence is gained from the observations of Yater et al.[4] made at autopsy of 950 soldiers dying of coronary artery disease, 47 per cent of whom were under age forty. Recently Enos, Holmes and Beyer[5] reported that 76.6 per cent of young soldiers in Korea who died suddenly of trauma had varying degrees of atherosclerosis in the coronary arteries. Although 48.3 per cent showed minor changes, 9.3 per cent had luminal narrowing greater than 50 per cent and 3 per cent had plaques causing complete occlusion of one or more vessels.

Diabetic. Still less is known of the frequency of coronary artery disease in living diabetic subjects. In an effort to assess its incidence at any and all stages, a large sample of hospitalized diabetic persons was carefully interviewed, examined and evaluated by one of us (J. B.) from October 17, 1953 to April 30, 1954. There were 394 diabetics, 217 women and 177 men. They were evaluated on or shortly after admission and no attempt at selection was made. Seventy-two (thirty-six women, thirty-six men) were age thirty or less, and another thirty were age forty or under.

Detailed observations will be presented subsequently[52] but, in summary, there were seventy-five persons with one or several manifestations of symptomatic coronary heart disease. This represented 19 per cent of the entire series and 25.5 per cent of those above age forty, since only one subject with heart disease was below this age. An additional eighty-six persons were strongly suspected of having heart disease of coronary origin by virtue of abnormal electrocardiograms. This 22 per cent with asymptomatic cardiac involvement added to those who had symptomatic heart disease gave a total incidence of over 40 per cent.

Data obtained at autopsies of diabetic subjects have demonstrated a 45 to 68 per cent incidence of marked coronary sclerosis or occlusion, while comparative figures for the same degree of atherosclerosis in unselected persons were only 8.2 to 29 per cent.[6–11] Using injection technics Stearns, Schlesinger and Rudy[12] obtained a higher rate of involvement, 74 per cent for the diabetic as against only 37 per cent for the non-diabetic. Warren and LeCompte[13] found that 69.8 per cent of all arteriosclerotic deaths in the diabetic involved the heart.

Fatal Coronary Heart Disease. Fatalities due to coronary heart disease have been reported from autopsy material in 19.6 to 35 per cent of diabetic subjects compared to an incidence of 2 to 22 per cent in the non-diabetic.[7,9,10,14] Again, the injection technic gave a higher incidence for both groups, 64 per cent in the diabetic and 23 per cent in unselected cases.[12] Warren and LeCompte[15] emphasized duration of diabetes, which averaged twelve years in 178 persons with fatal myocardial infarcts. Furthermore, of 157 recent fatal cases having diabetes at least fifteen years, 43 per cent died as a result of coronary atherosclerosis.[16] Clawson and Bell[2,17]

* From the Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts.

† Present address: Philadelphia General Hospital, Philadelphia, Pennsylvania.

also emphasized duration rather than severity of diabetes. They found fatal coronary disease twice as often in diabetic men and three times more frequently in diabetic women than in the non-diabetic population. Acceleration of occlusive coronary vascular disease has been well illustrated by Joslin and Wilson[18] who found fourteen deaths attributable to atherosclerosis involving the heart in 135 diabetic children, with onset of diabetes before age fifteen and an average duration of twenty-two years at the time of death.

Angina Pectoris. This symptom has been recorded in only 1.3 to 4.3 per cent of diabetics when outpatients have been surveyed[19] or clinical charts reviewed.[20] An incidence of 9 to 10 per cent has been most frequently reported[6,7,18,21] while other investigators[12] uncovered 28 per cent of diabetics and 15 per cent of non-diabetics with angina. The influence of diabetes is perhaps best summarized by Root and Sharkey:[7] "the frequency of angina pectoris trebles in the second ten years of diabetes." Data from the general population are meager. White[22] recorded 11.8 per cent in a special group with cardiac symptoms and signs.

Experience with the diabetic subject who has angina pectoris has been poor, although recent data in a large series are lacking. Whereas angina in the whole population has a lengthening survival[25–33] in both fatal (3 to 7.9 years) and surviving cases (4.6 to 18.4 years), it has augured death for the diabetic in an average of 1.6 years.[23] Of 136 fatal diabetic cases reported by Root and Graybiel[24] 52.5 per cent lived only a year or less, while nearly 85 per cent of the non-diabetic subjects reported by White,[30] Parker,[31] Block[33] and their associates survived the first year.

Myocardial Infarction. Myocardial infarction has affected 1.6 to 3.9 per cent of diabetic subjects, while the incidence for all cases has been 1 per cent or less.[41,42,44] The series of diabetics reported herewith numbered 160 among 11,393 hospitalized patients, or 1.4 per cent.

MORTALITY

Non-diabetic. No single figure can now be accurately applied to the over-all mortality from myocardial infarction or coronary occlusion. Earlier averages of 50 to 60 per cent when only more serious cases were considered have been reduced to a range of 15 to 25 per cent, according to Levine.[35] Recent uncomplicated first attacks have given the astonishingly low fatality rate of 3.4 per cent[36] to 8.5 per cent,[36–38] while 60 per cent mortality in "poor risk" patients is still recorded by Russek and Zohman.[36] The composite figures, however, indicate a persistent loss during acute attacks of 21.7 to 33.8 per cent.[36,39]

Diabetic. Mortality figures have shown less favorable results in most reports for both the first and for subsequent attacks. Death occurred in 39.3 per cent of diabetics and 27.7 per cent of non-diabetics studied by Master, Dack and Jaffe.[40] Most striking were the 51 per cent fatalities for diabetic subjects as compared to the 28 per cent rate of unselected cases reported by Katz et al.[42] and the 54 per cent of those dying among the series of 96 with acute infarcts reported by Root.[43] However, two recent series have indicated a less unfavorable experience with diabetes. Robinson[44] found that 40.8 per cent of his fifty-four diabetic patients died when all attacks were considered and only 30.8 per cent for the first infarct, while Cole et al.[45] recorded sixty-three cases with a 33.3 per cent of early loss from first attacks as compared to 27 per cent of 327 unselected cases.

Once the individual has lived two months following infarct, long-term survival of the diabetic subject has been found by Katz et al.[42] to be about equal to that of the non-diabetic, whereas Cole and others[45] reversed this finding by showing that diabetes did not affect the immediate outcome of the acute attack but did shorten long-term survival.

The increased number of women among diabetics suffering coronary occlusion has been consistently confirmed by many authorities. The usual ratio for a non-diabetic population in the range of two to four men to one woman has been strikingly reversed in the presence of diabetes, the incidence becoming about equal[7,8,10,12,40,42,45] for the sexes and at times has slightly favored the female.[6] To illustrate the effect of diabetes in another way Robinson[44] found that diabetes increased a woman's chances of incurring coronary occlusion by fourteen times. This report contrasts even more dramatically with non-diabetic experience when one considers coronary heart disease in patients under forty years of age. Gertler, Garn and White[46] found in such young subjects without hypertension or diabetes that men outnumbered women by twenty-four times. Ackerman et al.[47] in studying 600 women at autopsy have recently

shown significantly greater narrowing of coronary arteries in the twenty-five with diabetes.

It is obvious that more study of coronary artery disease in the diabetic is necessary if a further significant increase in life expectancy is to be accomplished. Myocardial infarction in a large hospitalized population with diabetes will be discussed in the present report. The striking total incidence of coronary artery disease in diabetic subjects has been mentioned. The fate of this latter group will subsequently be determined and should serve as a prognostic baseline for the evaluation of newer technics in treatment.

MATERIAL

The hospital charts of 160 persons who were known to have diabetes and who were in the New England Deaconess Hospital one or more times during the years 1943 to 1948 inclusive with a catalogued diagnosis related to myocardial infarction (coronary occlusion, coronary thrombosis, and the like) were studied. These dates were selected so that it would be possible in all cases to have at least a five year follow-up.

Fifty-eight cases were omitted from the study. Of these, thirty-one were eliminated because the diagnoses pertained to an old infarction and the remainder because of incorrect cataloguing or uncertainty concerning diagnosis. The 102 cases remaining were analyzed and followed until the patient's death or until completion of this survey. Diagnosis was established in all instances by serial electrocardiograms or autopsy findings or both.

With respect to follow-up, "acute mortality" was that concerned with any death occurring sixty days or less after the onset of symptoms, since under these circumstances death was related in a most direct fashion to the acute infarction. The "long term follow-up" was concerned with survival beyond the first more critical sixty days. In only two instances was information lacking after five years.

RESULTS

Acute Mortality. First and succeeding infarctions: There were 62 persons (24 men, 38 women) surviving less than sixty days after the onset of symptoms, an early mortality rate of 60.8 per cent. (Table I.) This mortality for all infarctions was two to three times that still recorded in unselected cases,[36,39,45] but was identical with that for the "poor risk" patients of Russek and Zohman.[36] It was even greater than the previous high acute mortalities of 39.3 to 54 per cent recorded in somewhat smaller series of studies of diabetics.[40,42–44]

Nineteen diabetics were known to have had a previous myocardial infarction. Fourteen failed to survive the present episode, a mortality of 74 per cent.

TABLE I

AGE, SEX AND ACUTE MORTALITY OF DIABETICS FOLLOWING MYOCARDIAL INFARCTION

Age	Male		Female		Total		Per cent Early Deaths
	No.	Early Deaths	No.	Early Deaths	No.	Early Deaths	
30–39	2	2	1	0	3	2	67
40–49	6	1	2	1	8	2	25
50–59	9	4	15	10	24	14	58
60–69	11	6	27	17	38	23	61
70–79	14	10	10	8	24	18	75
80–89	2	1	3	2	5	3	60
Total	44	24	58	38	102	62	60.8

First infarctions only: There were eighty-three patients in whom the attack represented an initial infarct. Of these forty-eight (or 57.8 per cent) died in sixty days or less. This early fatality rate exceeded greatly that of 31 per cent in Robinson's series[44] and 33 per cent recorded by Cole and others[45] for the first episode in their diabetic subjects.

Late Survival. After all infarctions: Table II shows the total 102 diabetic subjects, 30.4 per cent of whom survived for one year, 20.6 per cent for three years and 15.7 per cent for five years after the onset of acute myocardial infarction. These results were somewhat less favorable than those of Katz, Mills and Cisneros[42] who found that 50 per cent of their diabetic patients lived for one year, 33 per cent for three years and 20 per cent for five years. It should be further noted that survival beyond ten years is practically nil.

Following first attacks: On omission of the nineteen patients with known previous myocardial infarctions, the eighty-three remaining subjects were found to survive only slightly longer. Thus 32.5 per cent, 24.1 per cent and 19.3 per cent lived one, three and five years respectively. Again the experience was less favorable than that of Katz, especially in the interval of two months to one year following infarction, but the differences of average age

could explain in part the poorer results herein recorded.

Greatly improved long-term survivals have recently been noted by Cole, Singian and Katz[45] following first myocardial infarctions. For example, after five years 38.1 per cent of their diabetic patients and 49 per cent of unselected cases were alive. Furthermore, nearly one-half of the diabetic subjects in their five-year survival group were still alive at ten years, while only 3.6 per cent of the patients in the present series lasted a similar period. These late figures cannot be changed significantly, since only six persons were known to be living at the end of the current survey in 1954.

TABLE II
LONG-TERM SURVIVAL OF 102 DIABETICS FOLLOWING ACUTE MYOCARDIAL INFARCTION (1943 TO 1948, INCLUSIVE)

Survival Attained	Survivors		Living at end of Survey*	Incomplete Follow-up
	No.	Per cent		
Less than 60 days	62	60.8	0	..
60 days to 1 yr	40	39.2	0	1
1 yr	31	30.4	0	..
2 yr	27	26.5	0	..
3 yr	21	20.6	0	1
4 yr	17	16.7	0	..
5 yr	16	15.7	1	1
6 yr	11	10.8	4	..
7 yr	7	6.9	0	..
9 yr	6	5.9	1	..
10 yr	3	2.9	0	..
11 yr	1	1.0	0	..
12 yr	0	0	0	..
First Infarctions Only				
Less than 60 days	48	57.8	0	..
60 days to 1 yr	35	42.2	0	1
1 yr	27	32.5	0	..
3 yr	20	24.1	0	1
5 yr	16	19.3	1	1
10 yr	3	3.6	0	..

* Reports or examinations during 1954, February through October.

Cause of death: Of the forty survivors, twenty-five subsequently died of myocardial infarction or cardiac complications attributed to coronary artery disease. Six others died of miscellaneous causes, giving a total of thirty-one known dead.

FACTORS INFLUENCING OUTCOME

Sex. The general distribution (1.3 women to 1 man) favored women even beyond that expected as a result of the higher incidence of diabetes in women at fifty-five years of age and above. Twice the number of women as men of the same age (fifty to sixty-nine years of age) were affected, or 40 per cent of the entire series. The tendency for coronary artery disease to occur equally in diabetic men and women has been well established.[6–8,10,12,40,42,45] Among the small number of patients under the age of fifty in this study there were eight men and only three women. It is tempting to speculate that the factors outlined by Gertler and others[46] play some part in this sex distribution at a younger age.

The early mortality was greater in women as compared to men, 65.5 per cent as against 54.5 per cent. The difference was less striking than in Robinson's series of diabetic patients with coronary thrombosis[44] wherein 48.6 per cent of the women and 26.3 per cent of the men died. Review of the factors involved failed to show a significant effect from angina, hypertension, obesity, previous myocardial infarction or congestive heart failure. These are summarized in Table III.

Although hypertension was present in two women to one man, fewer women (60 per cent) than men (81.8 per cent) who died early were so affected. Furthermore, the fact that 60 per cent of the women who died within sixty days had none of these potential precipitating factors pointed directly to diabetes as the most important cause of the unfavorable prognosis.

Age. The average age of the entire group at the time of infarction was 63.0 years, for men 62.1 and women 63.4 years. It was not surprising that survivors of the acute attack were younger (average 60.6 years) than those who died early (average 64.5 years). Of the sixty-two persons comprising the acute mortality group, 89 per cent were from fifty to seventy-nine years old.

Since the patients in the current series were nearly five years older than the diabetic subjects reported by Cole[45] the higher mortality may thus be explained in part.

Duration of Diabetes Mellitus. The average duration of diabetes in the acute mortality group was 12.2 years, compared to 11.3 years in those surviving sixty days or more. No significant sex

TABLE III

ANALYSIS OF FACTORS INFLUENCING EARLY MORTALITY FOLLOWING MYOCARDIAL INFARCTION IN DIABETICS

Factor Studied	Female			Male			Total		
	Cases (no.)	Deaths (no.)	Mortality (%)	Cases (no.)	Deaths (no.)	Mortality (%)	Cases (no.)	Deaths (no.)	Mortality (%)
Angina	24	16	66.7	15	11	73.3	39	27	69.2
Hypertension	30	18	60.0	11	9	81.8	41	27	65.8
Previous myocardial infarction	14	10	71.4	5	4	80.0	19	14	73.7
Congestive heart failure	12	11	91.7	7	6	85.7	19	17	89.5
Obesity	28	20	71.4	13	8	61.5	41	28	68.2
Uncomplicated*	10	6	60.0	12	1	8.3	22	7	31.8

* Indicating the absence of angina, hypertension, previous myocardial infarction, congestive heart failure or obesity.

difference was noted. The average duration of diabetes for the 102 cases was 11.9 years. It is of interest to note that of three infarctions occurring under age forty, two had had diabetes for more than twenty-three years (the other for only seven years).

Control of Diabetes. Control of diabetes over the years is difficult to evaluate at best. No single group of criteria could readily be applied to these subjects, as only six were surviving and charts of the fatal cases were often prepared by different observers.

Insulin Dosage. The average insulin dosage in the acute mortality group was 29.4 units as compared to 26.3 units in the group surviving more than the first sixty days after onset of the first symptoms of infarction. Of forty-two persons taking less than twenty units of insulin per day, twenty-five or 59.5 per cent fell into the acute mortality group, a figure not differing significantly from the group as a whole.

Obesity. Of the sixty-two cases in the acute mortality group, twenty-eight (45 per cent) were grossly overweight. Thirteen of the forty (32.5 per cent) surviving beyond sixty days were obese. The acute mortality rate in those who were obese was 68.2 per cent. This suggests obesity, and the factors accompanying or perhaps responsible for it exert some influence in this group. Obesity was more common in diabetic women (50 per cent) than in men (30 per cent).

Hypertension. There were forty-one in all with hypertension (blood pressure above 150 mm. systolic or 90 mm. diastolic, or both) or 40.2 per cent. The incidence was twice as great in women (thirty of fifty-eight, 51.7 per cent) as in men (eleven of forty-four, 25.0 per cent). Twenty-seven (65.8 per cent) of those with hypertension died within sixty days, so that nearly one in two of those who died early had elevated blood pressure, while one in three of those surviving the acute episode also had hypertension. However, the early unfavorable effect was experienced chiefly by the men (Table III), of whom 82 per cent with hypertension failed to survive.

Pre-existing Angina Pectoris. Thirty-nine or 38.2 per cent of the entire group gave a history of angina. Of these, twenty-four were women and fifteen men, a ratio of 1.6:1. This predominance of women is a complete reversal of the usual 1:3.4 ratio of women to men with angina recorded by White[22] and Levine[35] for unselected cases. It is nearer the ratio for diabetic subjects of 1:1.4 (women to men) noted by Root and Graybiel[24] and of 1:1 in the series studied by Stearns.[12]

Evidence of pre-existing coronary artery disease in the form of angina was associated with a poorer prognosis since twenty-seven persons, or 69.2 per cent of those with the typical symptoms, did not survive the initial critical period. Thus 43.5 per cent of those who died early had angina, which had been present in only 30 per cent of the group still living after two months. Duration of angina pectoris prior to infarction could not be evaluated because of incomplete recording of this factor on the charts. It was also suspected that a considerably higher incidence of angina might have been found with earlier and more exhaustive notation of the patients' histories.

In the 390 unselected cases with first infarctions recently recorded by Cole and others,[45] angina was a premonitory symptom in 60 per cent.

Known Previous Myocardial Infarction. Nineteen of the entire group were known to have had previous myocardial infarction. This had occurred one year or less before the present episode in nine instances, one to five years earlier in seven, and its date was unknown in three. Fourteen, or 74 per cent, died early. Of the five survivors one was last reported alive in 1954, over nine years after the present attack. The other four died of another infarction from eight months to two and one-half years later.

Pre-existing Congestive Heart Failure. Nineteen persons (18.6 per cent) of the group had a history of congestive heart failure prior to the infarction under consideration. Of these, seventeen or almost 90 per cent did not survive beyond the first two months. Nine of those with congestive failure were also in the group with previous myocardial infarction.

Azotemia. The finding of a blood non-protein nitrogen level in excess of 40 mg. per cent at the time of infarction or subsequent thereto was associated with an increased mortality. Of forty-four persons with an elevated non-protein nitrogen level, thirty-five or almost 80 per cent failed to survive the initial critical period. In some cases serious renal disease pre-existed and undoubtedly exerted an unfavorable effect, while in others the elevated non-protein nitrogen level for the most part merely reflected the profoundly altered circulatory dynamics and heralded the fatal outcome.

Location of Infarction. Forty-eight infarctions were classified by electrocardiogram and/or by postmortem findings as chiefly anterior; an equal number were classified as chiefly posterior or inferior, while eight were combined or unlocalized. A study of the acute mortality and follow-up data does not reveal any significant effect of the location of the infarction upon survival.

Absence of Certain Pre-existing Complications. Of twenty-two persons in whom angina, hypertension, obesity, a history of heart failure or of previous infarction were absent, only seven or approximately 32 per cent died within the first sixty days. This is a percentage figure about one-half that of the group as a whole and approximates the mortality rate frequently reported for unselected cases.[36,39,40]

Most favorably affected by the absence of these factors were the men, of whom there were twelve, and only one death occurred (a mortality rate of 8.3 per cent). Early mortality for the women was still 60 per cent, again stressing the adverse effect of diabetes in women.

CHANGES IN THE DIABETIC STATE

The factors thus far evaluated in the production and outcome of myocardial infarction exerted their effect, as did the diabetes itself, with the passage of time. Appraisal of ketoacidosis and hypoglycemia before or in association with myocardial infarction gave some indication of the "acute" effects of diabetes.

Those with uncontrolled diabetes were divided into two groups: (1) Eleven having marked blood sugar elevations and acidosis with or without ketosis, and (2) an additional twenty-one subjects who had moderate hyperglycemia without acidosis and who required more insulin.

1. *Ketoacidosis.* Eleven diabetics (eight women and three men) averaging 67.4 years in age experienced marked blood sugar rises from 398 to 804 mg. per cent and acidosis indicated by reduction in blood CO_2 at levels of 9 to 25 mM. per liter. The majority showed ketonuria of sufficient degree to explain the lowered CO_2, and the two persons with a CO_2 of 9 mM./L. had the typical clinical picture of diabetic coma. Since blood ketone levels were not measured, acidosis occurring in one patient without ketonuria was unexplained. However, this patient had a blood non-protein nitrogen level of 140 mg. per cent and was virtually anuric. Ten of the eleven patients had definite peripheral vascular collapse prior to death, and in one its presence was rated as "probable."

No definite statement can be made as to the prior occurrence of acidosis or infarction for the series as a whole but in five instances acidosis appeared to come first, in three the infarction was the initial episode, and in three infarction and uncontrolled diabetes were simultaneous. Obviously, data from these critically ill persons could be misleading in regard to the sequence of events.

Most striking was that all eleven died, five within twenty-four hours. Postmortem examination in eight instances demonstrated extensive coronary atherosclerosis in addition to acute myocardial infarction. Sections from the liver have been carefully reviewed* and particular

* We are indebted to Dr. William Meissner, pathologist, New England Deaconess Hospital for review and interpretation of these sections.

attention paid to the presence or absence of liver cell necrosis. This was done because of the recent report of Ellenberg, Osserman and Pollack[49] indicating that hyperglycemia was a manifestation of "shock" and was brought about by central necrosis of liver cells.

TABLE IV
MYOCARDIAL INFARCTION: EFFECT ON INSULIN DOSAGE IN DIABETICS WITHOUT KETOACIDOSIS

Insulin Dose	No. of Cases	Fatal in 60 Days	Surviving
Unchanged	52	24	28
Increased	21	13	8
Decreased	7	3	4

With the exception of one instance in which a moderate degree of liver cell necrosis was seen, all of the current group showed merely the occasional falling out of cells and congestion occurring in any subject dying with cardiac failure. For comparison, a careful review of hepatic histology was made in twenty more patients who had not experienced ketoacidosis, but on whom autopsy had been performed. These included some with definite shock and some in whom this finding was not clinically demonstrable. Several from each group had experienced moderate to marked blood sugar increases prior to death. Again, only one showed liver cell changes regarded as necrosis, and in both the cytologic and architectural appearances could not be distinguished from those of the preceding eleven who had all experienced a more marked degree of hyperglycemia and who had acidosis. Furthermore, the patient with the highest blood sugar had no necrosis and no clinical evidence of shock.

2. *Increased Insulin Requirement.* Changes in insulin dosage during a period of seven days after the onset of symptoms heralding the myocardial infarction were arbitrarily regarded as significant when the dosage needed adjustment by at least 10 units. In eleven cases death occurred too soon for a change in insulin requirement to be evaluated. The remaining were distributed as in Table IV and do not include the previous eleven patients who had acidosis.

In some instances the increase was clearly related to the infarction. For example, patient C. A., a seventy year old woman was well standardized on 12 units of insulin daily. The infarct produced a rise in temperature, an elevated sedimentation rate and an insulin requirement of 48 units daily. During convalescence the dose was steadily decreased to a stable level of 8 units.

It was obvious that myocardial infarction did not always lead to uncontrolled diabetes. Furthermore, in some of the thirteen persons whose insulin dosage had to be increased data concerning the adequacy of diabetic control prior to infarction were incomplete. It was well appreciated that many diabetics hospitalized for any reason would require more insulin.

Shock was listed in twenty-four of the ninety-one having no acidosis, was probable in seven more, was unrecorded in two and could not be demonstrated in fifty-eight. Its occurrence for ten days in one man failed to produce acidosis, and the blood sugar was controlled by a total increase in insulin of only 14 units. On the other hand, another diabetic experienced a progressive increase in glycosuria requiring a sizable increment in insulin dosage but no evidence of shock was indicated. In two others who had the highest blood sugar values, shock was absent.

Hypoglycemia. Insulin-induced hypoglycemia has been shown to produce a variety of effects upon the heart and circulatory dynamics. A thorough summary of these has been prepared by Liebow and Hellerstein.[3] Earlier apprehension concerning the effect of low blood sugar on the metabolism of cardiac muscle have been allayed by the infrequent observation of myocardial infarction accompanying hypoglycemia[50] and the recent observations of Goodale and others[53] who found that insulin actually enhanced myocardial utilization of glucose in diabetic subjects down to blood sugar levels of 60 $\pm$ 10 mg. per cent. Current emphasis has shifted to the effects of epinephrine liberation after hypoglycemia and the consequent increase in cardiac work.

The present series failed to establish hypoglycemia as an important factor in the production of myocardial infarction. The patients were elderly on the average, yet careful search revealed only one instance in which hypoglycemia apparently led to a fatal occlusion. This was a sixty-four year old man who was taking 56 units of insulin daily. He experienced several reactions and about twelve hours before infarction was recognized he experienced a "very severe" reaction. At autopsy an occlusion in the posterior descending branch of the right

coronary artery was found. It is clear that hypoglycemia in itself did not produce such occlusion, but it is conceivable that the cardiac effects of epinephrine liberation following severe insulin reaction might have brought about such a change.

DISCUSSION

It is obvious that the diabetic subject in the present series did not do well when myocardial infarction developed, despite the somewhat higher average age than in the more favorable experiences noted by others.[42,45] A recent survey of diabetic subjects made during 1954 at the New England Deaconess Hospital indicated a persisting mortality greater than 50 per cent for those with acute infarctions. Thus diabetes has placed all these subjects in a "poor risk" category. Since treatment of the acute attack has followed the principles outlined by authorities reporting much lower mortalities in unselected cases, the incidence and extent of coronary artery disease in the diabetic person must account for this poor experience. Certainly data of the pathology have shown this to be so.

Although no data are available to prove that control of diabetes over the years will reduce or delay the development of coronary artery disease, evidence has accumulated to show that retinal, renal and peripheral vascular lesions occur less frequently and later in diabetic persons with good or excellent diabetic control.[54] Our own clinical impression is that early and continuing aggressive treatment of diabetes is the major factor in offsetting its adverse effect.

However, it is unlikely that even the best control of diabetes or the application of any metabolic principle known to date will reverse coronary changes already present. The greater than 40 per cent incidence of coronary artery disease found in a large sample of a hospitalized diabetic population, nearly 20 per cent of which was under thirty years of age, indicates a reasonable site for future attack, whether by medical or surgical means. Follow-up of this group will eventually contribute much to the knowledge of this problem.

The uniformly fatal outcome in those with uncontrolled diabetes and acidosis is too striking to avoid comment. Similar unfavorable results were stressed by Katz[42] who found a 56.2 per cent mortality rate for diabetic subjects who had ketosis and only a 27.2 per cent rate when this finding was absent.

That diabetic acidosis has a profound effect in producing peripheral vascular collapse is well known and an excellent summary of the data substantiating the grave prognosis of such "shock" has been prepared by Liebow and Hellerstein.[3] It is reasonable to suppose that the diabetic with coronary atherosclerosis may suffer myocardial infarction when the dehydration, hemoconcentration and hypotension of severe acidosis are added.

However, the effects of peripheral vascular failure from any severe, acute medical illness have produced hyperglycemia according to Davidson and others.[48] They also found varying degrees of acidosis without ketosis, which could in part be explained by increased serum levels of lactic acid. Acute central liver cell necrosis was demonstrated at autopsy in some cases. From these data the possibility was entertained that advanced liver failure produced hyperglycemia because of increased glycogenolysis and gluconeogenesis.

Ellenberg, Osserman and Pollack[49] summarized reports of hyperglycemia and glycosuria discovered at the time of myocardial infarction. These have indicated that abnormal carbohydrate metabolism either represented diabetes mellitus uncovered by the acute attack or transitory hyperglycemia reversed completely with recovery from the infarct In an autopsy series of sixty non-diabetic subjects and fifteen diabetics with myocardial infarction these authors re-affirmed the effect of severe shock in producing hyperglycemia in the group of non-diabetic subjects and in causing uncontrolled diabetes, often with ketoacidosis, among the diabetics. In all subjects who had undergone "shock" they found central liver cell necrosis, to which they attributed the changes in carbohydrate metabolism. Weinstein[51] reported two diabetics in whom myocardial infarction was heralded by increased hyperglycemia and glycosuria.

The present study did not confirm the occurrence of liver cell necrosis in a significant number of the eleven diabetic persons, or in sufficient degree when it was found to explain the marked blood sugar and CO_2 alterations observed. However, definite histologic changes were noted. Whether these could indicate enough derangement in the handling of carbohydrate and other metabolites by the liver to explain the blood findings is conjectural. Poor correlation between the presence or absence of

shock, blood sugar levels and liver histology, indicated that some factor or factors not directly connected with the microscopic findings in the liver was at work. The clinical behavior of diabetes in those persons with marked hyperglycemia only and who subsequently recovered was more like that seen in diabetics during infection.

SUMMARY

1. Coronary artery disease has in recent years produced nearly one-half of all deaths in diabetic patients.

2. Preliminary observations concerning the incidence of coronary artery disease in a large sample of hospitalized diabetic patients indicate its presence in over 40 per cent.

3. The presence of diabetes mellitus has been associated with high mortality from acute myocardial infarction: 60.8 per cent following all attacks and 57.8 per cent after the first attack. Thus the diabetic with acute myocardial infarction has a prognosis similar to that of "poor risk" cases from the general population.

4. The high incidence of and mortality from myocardial infarction in diabetic women contributed measurably to the over-all poor experience.

5. That diabetes *per se* played a major part in early mortality, particularly as a result of its effect upon women, was shown by the 60 per cent early fatality rate for the series of women who did not have angina, hypertension, obesity, heart failure or previous myocardial infarction. On the other hand, an early mortality of 8.3 per cent was found for men in whom these factors were absent.

6. Late survival of diabetic patients after the initial attack of myocardial infarction was also decreased, since fewer than 20 per cent lived five years and only 3.6 per cent for ten years.

7. Early death occurred in all of eleven diabetic subjects having marked hyperglycemia and acidosis with or without ketosis. Although each experienced "shock," it could not be shown that changes in carbohydrate metabolism were directly related to the presence of shock or to histologic changes in the liver at autopsy.

REFERENCES

1. Joslin, E. P., Root, H. F., White, P. and Marble, A. Treatment of Diabetes Mellitus, 9th ed., p. 187. Philadelphia, 1952. Lea & Febiger.
2. Clawson, B. J. and Bell, E. T. Incidence of fatal coronary disease in non-diabetic and in diabetic persons. *Arch. Path.*, 48: 105, 1949.
3. Liebow, I. M. and Hellerstein, H. K. Cardiac complications of diabetes mellitus. *Am. J. Med.*, 7: 660, 1949.
4. Yater, W. M., Welsh, P. P., Stapleton, J. F. and Clark, M. L. Comparison of clinical and pathologic aspects of coronary artery disease in men of various age groups: a study of 950 autopsied cases from the Armed Forces Institute of Pathology. *Ann. Int. Med.*, 34: 352, 1951.
5. Enos, W. F., Holmes, R. H. and Beyer, J. Coronary disease among United States soldiers killed in action in Korea. *J. A. M. A.*, 152: 1090, 1953.
6. Blotner, H. Coronary disease in diabetes mellitus. *New England J. Med.*, 203: 709, 1930.
7. Root, H. F. and Sharkey, T. P. Coronary arteriosclerosis in diabetes mellitus. *New England J. Med.*, 215: 605, 1936.
8. Nathanson, M. H. Disease of the coronary arteries. Clinical and pathological features. *Am. J. M. Sc.*, 170: 240, 1925.
9. Root, H. F., Bland, E. F., Gordon, W. H. and White, P. D. Coronary atherosclerosis in diabetes mellitus. *J. A. M. A.*, 113: 27, 1939.
10. Lisa, J. R., Magiday, M., Galloway, I. and Hart, J. F. Arteriosclerosis with diabetes mellitus. A study of the pathologic findings in 193 diabetic and 2,250 nondiabetic patients. *J. A. M. A.*, 120: 192, 1942.
11. Millard, E. B. and Root, H. F. Degenerative vascular lesions and diabetes mellitus. *Am. J. Digest. Dis.*, 15: 41, 1948.
12. Stearns, S., Schlesinger, M. J. and Rudy, A. Incidence and clinical significance of coronary artery disease in diabetes mellitus. *Arch. Int. Med.*, 80: 463, 1947.
13. Warren, S. and LeCompte, P. M. The Pathology of Diabetes Mellitus, 3rd ed., p. 138. Philadelphia, 1952. Lea & Febiger.
14. Enklewitz, M. Diabetes and coronary thrombosis. An analysis of cases which came to necropsy. *Am. Heart J.*, 9: 386, 1934.
15. See reference 13, p. 158.
16. See reference 13, p. 220.
17. Bell, E. T. A postmortem study of 1,214 diabetic subjects with special reference to the vascular lesions. *Proc. Am. Diabetes A.*, 10: 62, 1950.
18. Joslin, E. P. and Wilson, J. L. Lessons for future treatment from 472 fatalities in diabetic children. *Brit. M. J.*, 2: 1293, 1950.
19. Rabinowitch, I. M., Ritchie, W. L. and McKee, S. H. A statistical evaluation of different methods for the detection of arteriosclerosis in diabetes mellitus. *Ann. Int. Med.*, 7: 1478, 1934.
20. See reference 1, p. 421.
21. Friedman, G. Cardiovascular status of diabetics after the fourth decade of life. *Arch. Int. Med.*, 55: 371, 1935.
22. White, P. D. Heart Disease, 3rd ed. New York, 1944. Macmillan Co.
23. See reference 1, p. 430.
24. Root, H. F. and Graybiel, A. Angina pectoris and diabetes mellitus. *J. A. M. A.*, 96: 925, 1931.

25. Herrick, J. B. and Nuzum, F. R. Angina pectoris. Clinical experience with 200 cases. *J. A. M. A.*, 70: 67, 1918.
26. White, P. D. Prognosis of angina pectoris and of coronary thrombosis. *J. A. M. A.*, 87: 1525, 1926.
27. White, P. D. and Bland, E. F. Further report on the prognosis of angina pectoris and of coronary thrombosis. Study of 500 cases of former condition and of 200 cases of latter. *Am. Heart J.*, 7: 1, 1931.
28. Eppinger, E. C. and Levine, S. A. Angina pectoris. Some clinical considerations with special reference to prognosis. *Arch. Int. Med.*, 53: 120, 1934.
29. Wedd, A. M. and Smith, R. E. Observations on prognosis of angina pectoris. *Am. J. M. Sc.*, 189: 690, 1935.
30. White, P. D., Bland, E. F. and Miskall, E. W. Prognosis of angina pectoris. Long time follow-up of 497 cases including note on 75 additional cases of angina pectoris decubitus. *J. A. M. A.*, 123: 801, 1943.
31. Parker, R. L., Dry, T. J., Willius, F. A. and Gage, R. P. Life expectancy in angina pectoris. *J. A. M. A.*, 131: 95, 1936.
32. Sigler, L. H. Prognosis of angina pectoris and coronary occlusion. Follow-up of 1,700 cases. *J. A. M. A.*, 146: 998, 1951.
33. Block, W. J., Crumpacker, E. L., Dry, T. J. and Gage, R. P. Prognosis of angina pectoris. *J. A. M. A.*, 150: 259, 1952.
34. Zoll, P. M., Wessler, S. and Blumgart, H. L. Angina pectoris: clinical and pathological correlation. *Am. J. Med.*, 11: 331, 1951.
35. Levine, S. A. Clinical Heart Disease, 4th ed., p. 116. Philadelphia, 1951. W. B. Saunders Co.
36. Russek, H. I. and Zohman, B. L. Chances for survival in acute myocardial infarction. *J. A. M. A.*, 156: 765, 1954.
37. Master, A. M., Jaffe, H. L. and Dack, S. The treatment and immediate prognosis of coronary artery thrombosis (267 attacks). *Am. Heart J.*, 12: 549, 1936.
38. Baer, S., Krasnoff, S. O. and Heine, W. I. Criteria for the use of anticoagulants in acute myocardial infarction. *Pennsylvania M. J.*, 56: 274, 1953.
39. Smith, F. J., Keyes, J. W. and Denham, R. M. Myocardial infarction: a study of the acute phase in 920 patients. *Am. J. M. Sc.*, 221: 508, 1951.
40. Master, A. M. Dack, S. and Jaffe, H. L. Age, sex and hypertension in myocardial infarction due to coronary occlusion. *Arch. Int. Med.*, 64: 767, 1939.
41. See reference 1, p. 418.
42. Katz, L. N., Mills, G. Y. and Cisneros, F. Survival after recent myocardial infarction. *Arch. Int. Med.*, 84: 305, 1949.
43. See reference 1, p. 507.
44. Robinson, J. W. Coronary thrombosis in diabetes mellitus. *New England J. Med.*, 246: 332, 1952.
45. Cole, D. R., Singian, E. B. and Katz, L. N. The long-term prognosis following myocardial infarction, and some factors which affect it. *Circulation*, 9: 321, 1954.
46. Gertler, M. M., Garn, S. M. and White, P. D. Young candidates for coronary heart disease. *J. A. M. A.*, 147: 621, 1951.
47. Ackerman, R. F., Dry, T. J. and Edwards, J. E. Relationships of various factors to degree of atherosclerosis in women. *Circulation*, 1: 1345, 1950.
48. Davidson, C. S., Lewis, J. H., Tagnon, H. J., Adams, M. A. and Taylor, F. H. L. Medical shock: abnormal biochemical changes in patients with severe acute medical illnesses, with and without peripheral vascular failure. *New England J. Med.*, 234: 279, 1946.
49. Ellenberg, M., Osserman, K. E. and Pollack, H. Hyperglycemia in coronary thrombosis. *Diabetes*, 1: 16, 1952.
50. See reference 1, p. 426.
51. Weinstein, A. Coronary thrombosis and diabetes mellitus: the influence of coronary thrombosis on the metabolism of the diabetic patient. *Am. J. Digest Dis.*, 18: 287, 1951.
52. Bryfogle, J. W. and Bradley, R. F. To be published.
53. Goodale, W. T., Olson, R. E. and Hackel, D. B. The effect of mild diabetes and insulin on heart muscle metabolism in man. *J. Clin. Investigation*, 30: 642, 1951.
54. Wilson, J. L., Root, H. F. and Marble, A. Prevention of degenerative vascular lesions in young patients by control of diabetes. *Am. J. M. Sc.*, 221: 479, 1951.

Production of Renal Ischemia and Proteinuria in Man by the Adrenal Medullary Hormones*

S. Edward King, M.D.† *and* Capt. David S. Baldwin, M.C., U. S. Army†
New York, New York

Manifestations of vasomotor and emotional instability are commonly seen in individuals with transient or intermittent orthostatic proteinuria.‡[1-4] Conversely, proteinuria has been demonstrated in normal subjects following psychogenic stimuli,[5] exposure to cold[6,7] and erect immobilization in tilt or lordosis,[8] renal vasoconstriction being common to all.[9]

Sweating, pallor and variations in pulse and blood pressure, as well as the intense renal vasoconstriction which appears under these conditions, suggest sympathetic excitation. Although association of renal vasoconstriction and proteinuria is frequently cited, the sole direct evidence implicating the sympathomimetic amines is based on work of Starr with intravenous epinephrine in rabbits and dogs, and oral and subcutaneous ephedrine in man.[10]

Conspicuous vasomotor lability in individuals with intermittent proteinuria suggested renal ischemia as the underlying mechanism, possibly mediated through the adrenal medullary hormones. Accordingly, the production of proteinuria by intravenous L-nor-epinephrine and epinephrine in man was attempted. Proteinuria has been induced consistently in this manner.

METHOD

Observations of glomerular filtration rate (GFR) as measured by inulin clearance, renal plasma flow (RPF) as measured by p-aminohippurate clearance, urine volume rate (V) and protein excretion were made in six normal young adult males and six additional subjects with a history of intermittent orthostatic proteinuria. The effect of L-nor-epinephrine was determined in ten instances and epinephrine (U.S.P.) in four. All subjects were studied recumbent, in a basal hydropenic state after fifteen hours of fluid restriction. Urine was collected by indwelling catheter. Qualitative tests for protein were performed on small samples of catheter urine at five- to ten-minute intervals.

Three control periods, each of approximately fifteen minutes' duration, preceded intravenous administration of the pressor amines. L-Nor-epinephrine was infused by constant infusion pump at rates varying from 15 to 44 μg./min. for sixteen to forty-five minutes in ten different individuals, the rate usually being increased when proteinuria failed to appear with initial dosage. Epinephrine was infused in a similar manner at rates varying from 15 to 25 μg./min. for periods ranging from twenty-four to thirty-three minutes. Observations were continued for twenty-three to fifty-four minutes after discontinuance of administration of the amines.

The maximal tubular reabsorptive rate for glucose (T_mG) was determined in five additional normal young adult male subjects with intravenous L-nor-epinephrine infused at a constant rate of 30 μg./min.

Inulin was determined by a modification of Harrison's method;[11] p-aminohippurate by the method of Smith, Finkelstein, Aliminosa, Crawford and Graber.[12] Urinary protein was determined qualitatively by 20 per cent sulfosalicylic acid, which is considered sensitive to concentrations of protein exceeding 10 mg. per cent.[13] Glucose was determined by Nelson's modification of the Somogyi method.[14] Pulse rates and sphygmomanometric blood pressures were taken at five-minute intervals throughout the period of study. Mean arterial blood pressure was estimated as the sum of one-third systolic and two-thirds diastolic pressure.

RESULTS

Proteinuria. L-nor-epinephrine: Intravenous L-nor-epinephrine caused proteinuria in eight of

‡ Transient or intermittent proteinuria is a term used here to indicate the *irregular* appearance of protein in the urine during the erect posture as opposed to *regularly* occurring postural proteinuria. The former is believed to be related to vasomotor instability, while the latter may be anatomic in origin.

* From the Renal Research Laboratory, First Army, U. S. Army Hospital, Fort Jay, New York.
† Present address: Bellevue Medical Center, New York University.

TABLE 1

EFFECT OF INTRAVENOUS L-NOR-EPINEPHRINE ON RENAL HEMODYNAMICS, URINE FLOW AND PROTEINURIA

Subject	Body Surface Area (sq. M.)	Period	Duration (min.)	L-Nor-epinephrine (μg./min.)	GFR (cc./min. per 1.73 sq. M.) §§	RPF (cc./min. per 1.73 sq. M.) §§	F.F.§§	Urine Volume (cc./min.)	Urine Protein‡‡	Pulse Rate*	Blood Pressure† (mm. Hg)
1 (P)§	2.26	Control**	61	..	132	522	0.25	0.93	0	66	135/85
		Nor-epinephrine	27	15	91	322	0.28	0.65	++	48	194/114
		Recovery	50	..	96	368	0.26	0.50	++	70	136/70
		Recovery	8	..	90	400	0.23	0.64	0	...	.....
2 (P)	1.65	Control**	46	..	116	585	0.20	0.84	0	75	110/70
		Nor-epinephrine	33	25	96	342	0.28	0.45	0	60	174/118
		Recovery	25	..	133	646	0.21	0.55	0	102	116/68
3 (P)	2.06	Control**	40	..	136	659	0.21	1.49	0	72	128/80
		Nor-epinephrine	10	25	93	368	0.25	1.25	0	54	152/98
		Nor-epinephrine	21	42	80	299	0.27	0.42	++	54	190/98
		Recovery	46	..	...	...	....	.....	++	90	122/60
		Recovery	14	..	112	569	0.20	0.42	0	...	.....
4 (P)	1.93	Control**	41	..	135	544	0.25	0.59	0	60	120/72
		Nor-epinephrine	35	34	92	272	0.34	0.32	0	45	198/110
		Recovery	48	..	...	...	....	.....	+	84	122/70
5 (P)	1.84	Control**	38	..	123	576	0.21	0.58	0	72	102/50
		Nor-epinephrine	27	25	130	482	0.27	2.00‡	Trace	60	162/80
		Nor-epinephrine	13	42	112	320	0.35	3.47‡	Trace	69	178/82
		Recovery	23	..	108	505	0.21	5.11‡	Trace	88	130/70
6 (N)††	2.20	Control**	44	..	122	531	0.23	0.66	0	69	122/72
		Nor-epinephrine	28	22	105	282	0.38	0.47	0	48	144/80
		Nor-epinephrine	17	44	47	116	0.41	0.21	0	51	170/72
		Recovery	13	..	120	487	0.25	0.68	+	...	.....
7 (N)	1.76	Control**	38	..	118	517	0.23	0.89	0	69	134/60
		Nor-epinephrine	16	25	105	242	0.43	0.90	++	50	204/104
		Recovery	53	..	125	464	0.28	0.86	++	80	134/58
8 (N)	2.10	Control**	34	..	140	591	0.24	1.38	0	75	134/66
		Nor-epinephrine	39	25	120	392	0.31	0.70	0	45	198/110
		Recovery	24	..	...	...	....	.....	0	78	142/60
9 (N)	1.81	Control**	45	..	111	604	0.18	1.34	0	78	142/40
		Nor-epinephrine	11	15	103	498	0.21	0.80	Trace	81	164/80
		Nor-epinephrine	17	34	72	281	0.26	0.87	Trace	84	198/86
		Recovery	30	..	103	636	0.16	1.08	Trace	93	144/60
10 (N)	2.05	Control**	47	..	133	686	0.20	1.20	0	81	120/60
		Nor-epinephrine	28	32	69	274	0.25	0.47	Trace	57	176/84
		Recovery	52	..	126	699	0.18	0.92	+	96	130/76

* Slowest pulse rates observed are recorded for L-nor-epinephrine periods.

† Highest systolic blood pressures observed are recorded for L-nor-epinephrine periods.

‡ Diuresis following water load (not included in average value for volume).

§ (P) = History of intermittent orthostatic proteinuria.

** Average of three periods.

†† (N) = Normal subjects.

‡‡ Quantitative protein excretion (biuret) in timed specimens was determined, but frequent qualitative testing provided a more sensitive index of maximum intensity of proteinuria. Concentrations reaching 0.29 gm. % were observed.

§§ The differences in mean value for GFR, RPF and FF (filtration fraction) between control and nor-epinephrine periods are statistically significant ($p < .001$).

ten subjects. There was no difference in the response of normal subjects and those with intermittent proteinuria. The onset of proteinuria occurred within nine to thirty-one minutes at infusion rates of 15 to 44 μg./min. The duration of proteinuria varied from twenty-five to fifty-three minutes and persisted for sixteen to fifty minutes after the infusion was discontinued. The maximal degree of proteinuria varied from a trace to ++, usually occurring within twenty minutes after the onset of proteinuria.

No direct relationship was evident between the rate or duration of L-nor-epinephrine infusion and the occurrence, degree and duration of proteinuria. However, in two subjects in whom proteinuria failed to appear with 25 μg., it was induced by 42 μg./min. Infusion rates of only 25 μg./min. were employed in the two instances in which proteinuria did not occur.

Although catheter samples of urine usually were tested qualitatively for protein at five-minute intervals, in three subjects only pooled clearance period specimens were examined. The degree of proteinuria observed in the latter subjects may not represent the maximal intensity.

Epinephrine (U.S.P.): Intravenous epinephrine caused proteinuria in two normal subjects and two with intermittent orthostatic proteinuria. The onset of proteinuria occurred within eight to thirty-six minutes at infusion rates of 15 or 25 μg./min. The duration varied from

TABLE II

EFFECT OF INTRAVENOUS EPINEPHRINE ON RENAL HEMODYNAMICS, URINE FLOW AND PROTEINURIA

Subject	Body Surface Area (sq. M)	Period	Duration (min.)	Epinephrine (μg./min.)	GRF (cc./min. per 1.73 sq. M.)	RPF (cc./min. per 1.73 sq. M.)	F.F.	Urine Volume (cc./min.)	Urine Protein	Pulse Rate*	Blood Pressure† (mm. Hg)
1 (P)‡	1.93	Control**	41	..	135	544	0.25	0.59	0	60	120/72
		Epinephrine	25	21	71	215	0.33	0.34	+	87	190/60
		Recovery	54	..	83	306	0.27	0.27	+	84	118/74
2 (P)	1.80	Control**	41	..	115	581	0.20	0.76	0	84	118/56
		Epinephrine	29	15	102	301	0.34	0.39	0	111	158/0
		Recovery	46	..	117	491	0.24	0.35	Trace	78	134/70
		Recovery	29	..	....	....	....	....	0	93	126/70
3 (N)§	1.81	Control**	45	..	111	604	0.18	1.34	0	78	142/40
		Epinephrine	33	22	69	290	0.24	0.80	Trace	114	170/0
		Recovery	31	..	78	394	0.20	0.52	Trace	84	136/50
4 (N)	1.55	Control**	44	..	98	520	0.19	0.77	0	84	112/80
		Epinephrine	24	15	69	288	0.24	0.46	+	84	180/76
		Recovery	44	..	86	398	0.21	0.38	Trace	114	116/76

* Maximal pulse rates observed are recorded for epinephrine periods.
† Highest pulse pressures observed are recorded for epinephrine periods.
‡ (P) = History of intermittent orthostatic proteinuria.
§ (N) = Normal subjects.
** Average of three periods.

thirty-two to seventy-two minutes, proteinuria persisting for seven to forty-eight minutes after discontinuation of the infusion. The maximal degree of proteinuria varied from a trace to ++ and occurred within thirty minutes after the onset of proteinuria. In two instances proteinuria was induced by epinephrine at a rate of 25 μg./min. after 15 μg. had proved ineffective.

Proteinuria solely attributable to concentration of urine following the amines is excluded by the failure of initial low rates of urine flow to be further depressed to a marked degree. Microscopic examination of fresh urine sediment failed to reveal hematuria or other significant abnormalities.

Renal Hemodynamics (Tables I and II; Figs. 1 to 4). *L-nor-epinephrine:* Rates of L-nor-epinephrine of 25 μg./min. or less (15 to 25 μg.) were employed in eight instances. RPF was reduced in all. The average RPF was decreased 36 per cent from 573 cc./min. to 366 cc./min. GFR was depressed by L-nor-epinephrine in six of the eight observations. The average GFR decreased 16 per cent, from 125 cc./min. to 105 cc./min. The filtration fraction (FF) was increased in all instances. The average FF increased 36 per cent, from 0.22 to 0.30.

L-nor-epinephrine was administered in six instances at more rapid rates (32 to 44 μg./min.). RPF was decreased in all. The average RPF was reduced 57 per cent, from 600 cc./min. to 260 cc./min. GFR fell in all instances. The average GFR was decreased 38 per cent, from 127 cc./min. to 79 cc./min. FF was increased in all. The average FF increased 48 per cent, from 0.21 to 0.31.

Epinephrine (U.S.P.): Epinephrine was administered intravenously at rates of 15 to 22 μg./min. in four subjects. RPF and GFR were

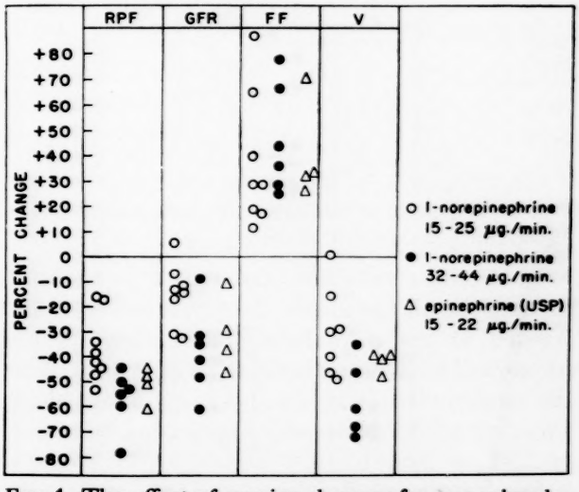


FIG. 1. The effect of varying dosage of L-nor-epinephrine and epinephrine (U.S.P.) on renal plasma flow, glomerular filtration rate, filtration fraction and urine volume. With higher doses of L-nor-epinephrine there is greater depression of RPF, GFR and V. The effects of epinephrine approximate those of the larger L-nor-epinephrine doses.

TABLE III

ALTERATIONS IN RENAL VASCULAR RESISTANCE FOLLOWING INTRAVENOUS L-NOR-EPINEPHRINE AND EPINEPHRINE (U.S.P.)

	Control	Nor-epinephrine (15–25 μg./min.)	Control	Nor-epinephrine (32–44 μg./min.)	Control	Epinephrine (15–22 μg./min.)
Total renal resistance*.....	5.77	12.92	5.38	17.60	5.84	11.47
Afferent resistance*.....	1.97	6.62	1.38	9.50	1.77	3.94
Net efferent resistance*.....	1.56	2.54	1.87	2.69	1.81	2.51
Venular resistance*.....	2.24	3.76	2.13	5.41	2.26	5.02

* Dynes. Sec. Cm.$^{-5}$ × 10^3.

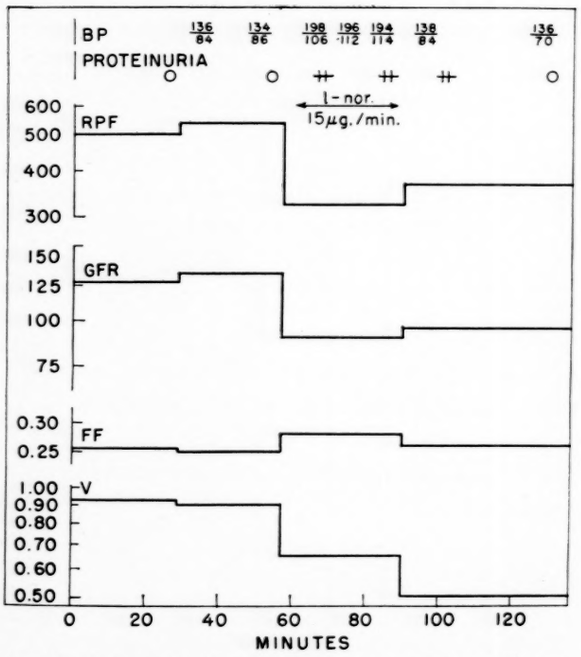


FIG. 2. The effect of intravenous L-nor-epinephrine at 15 μg./min. in subject 1 (Table I).

reduced in all instances. The average RPF decreased 51 per cent, from 562 cc./min. to 274 cc./min. The average GFR fell 32 per cent, from 115 cc./min. to 78 cc./min. FF increased in all. The average FF increased 43 per cent, from 0.21 to 0.30.

Renal Vascular Resistances. Renal resistances were calculated according to the formulas of Gomez.[15] Normal values were assumed for venous pressure during control and experimental periods. Although L-nor-epinephrine and epinephrine may increase systemic venous pressure moderately,[16,17] such variations would not in these instances influence the calculations significantly.

TABLE IV

THE EFFECT OF INTRAVENOUS L-NOR-EPINEPHRINE (30 μg./min.) ON T_m GLUCOSE

Subject	Body Surface Area (sq. M.)	T_{mG}*		
		Control† (mg./min.)	L-Nor-epinephrine† (mg./min.)	Per cent Change
1	1.86	501	499	0
2	1.95	305	328	+6
3	1.35	305	330	+12
4	1.84	352	304	−13
5	1.78	340	346	−2

* Corrected for surface area to 1.73 sq. M.
† Average of three periods.
Note: Proteinuria was not demonstrable with urinary dilution due to glucose diuresis (V. > 10 cc./min.).

Mean alterations of total and component resistance at infusion rates of 25 μg./min. or less L-nor-epinephrine, 32 to 44 μg./min. L-nor-epinephrine and 15 to 22 μg/min. epinephrine (U.S.P.) are presented in Table III.

Maximal Tubular Reabsorptive Capacity for Glucose (T_mG). The effect of L-nor-epinephrine on the maximal rate of tubular reabsorption of glucose was determined in five subjects. No appreciable effect on this function was observed. (Table IV.)

Rate of Urine Flow (Tables I and II; Figs. 1 to 4). In the eight instances in which L-nor-epinephrine was administered at a rate of 25 μg./min. or less, the urine volume was diminished

in all but one. The average urine flow per minute decreased 36 per cent, from 1.08 cc./min. to 0.75 cc./min. The urine volume was reduced in all six instances in which over 25 μg./min. of L-nor-epinephrine was administered. The average urine flow decreased 57 per cent, from 1.06 cc./min. to 0.46 cc./min.

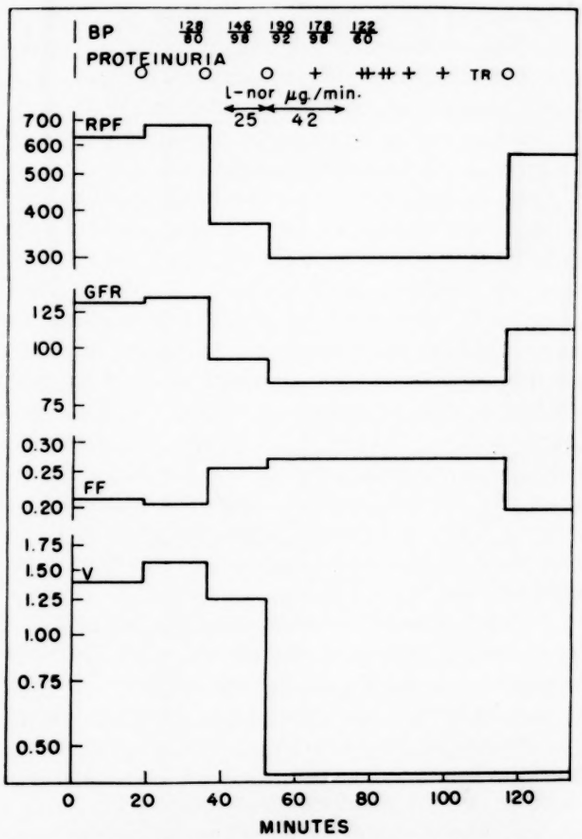


FIG. 3. The effect of intravenous L-nor-epinephrine at doses of 25 and 42 μg./min. in subject 3 (Table I).

The urine volume was diminished by epinephrine in all four instances. The average urine flow decreased 42 per cent, from 0.87 cc./min. to 0.50 cc./min.

COMMENTS

No untoward symptoms or signs followed the intravenous administration of L-nor-epinephrine at the rates employed. Slight headache occurred in several instances. Facial pallor was usual. Systolic and diastolic hypertension were associated with bradycardia. (Table I.) The mean arterial pressure was increased in all instances. Symptoms were more conspicuous following intravenous epinephrine. Sweating, tremulousness and palpitation with pallor were usual. Elevation in systolic blood pressure with variable reduction in diastolic pressure and tachycardia occurred.

These studies demonstrate that transient proteinuria of short duration and varying intensity can be induced with regularity in man

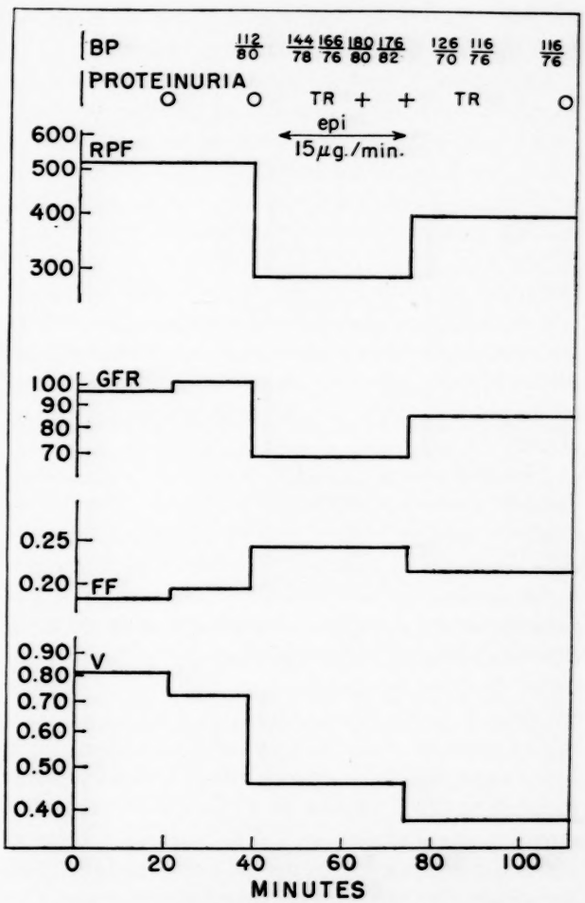


FIG. 4. The effect of intravenous epinephrine (U.S.P.) at 15 μg./min. in subject 4 (Table II).

following intravenous administration of the adrenal medullary amines, L-nor-epinephrine and epinephrine. The observations of Starr[10] relating proteinuria to presumed renal vasoconstriction were limited to epinephrine in rabbits and dogs and ephedrine in man. Addis, Barrett, Boyd and Ureen[18] failed to induce proteinuria in rats with epinephrine. Subcutaneous epinephrine in a dosage of 1 mg. in man does not produce this effect.*

* We were unable to produce proteinuria in five subjects in whom moderate renal ischemia was induced by 1 mg. of epinephrine (U.S.P.) administered subcutaneously.

Proteinuria, although transient, may occur at significant rates. The rates of protein excretion were determined quantitatively in timed specimens collected for renal clearance measurements. In view of the low urine flow these collections often extended for periods beyond the duration of intense proteinuria. Consequently such values do not reflect the maximal rate of protein excretion. Excretion rates as high as 1.48 mg./min. were nevertheless observed.

Detection of proteinuria in this study may be ascribed to several conditions of the protocol which was planned with this objective. Hydropenia, with resultant low urine flow in contrast to diuresis customarily employed in clearance studies, provided ideal circumstances for demonstration of small amounts of urinary protein. Frequent sampling of urine permitted recognition of transient proteinuria which might otherwise not have been detected in pooled specimens. Finally, the rate of infusion was increased in most instances when proteinuria failed to appear.

The reduction in RPF observed with intravenous L-nor-epinephrine and epinephrine in dosage varying from 15 to 44 μg./min. exceeds that reported by others[19–21] with smaller dosage.

Similarly, the reduction in GFR noted in the present study, which is in marked contrast with the unchanged GFR previously observed in man[19,20] may be ascribed to the higher dosage employed here. Pullman and McClure[21] obtained marked reduction in GFR in one instance at a rate of L-nor-epinephrine infusion comparable to ours. Moyer and Handley[22] recorded significant reduction in GFR in dogs receiving a dosage which, in terms of body weight, was five times the dosage employed by us.

Nickel, Smythe, Papper and Bradley reported that in subjects with a previous water load, urine flow usually remains unchanged or increases following intravenous medullary amines despite decreased sodium excretion.[23] They attribute this finding to possible suppression of antidiuretic hormone by the amines. Reduction in urine flow observed in the present study is related to several factors. Diminished glomerular filtration rate could account significantly for this finding. However, augmented tubular reabsorption of water also occurred, as evidenced by increased inulin U/P ratios, which is probably an osmotic effect resulting from decreased electrolyte excretion. Furthermore, it is likely that any presumed diuretic action of the medullary hormones through suppression of antidiuretic hormone was excluded in our studies by the strict hydropenic conditions imposed.

Calculations of renal resistance indicate that with graduated doses of L-nor-epinephrine there is increase in all components. The predominant effect is a marked increase in afferent resistance. With larger doses there is no further effect upon net efferent resistance whereas there are progressive increments in afferent resistance and to a lesser degree in venular resistance. With epinephrine there appears to be a relatively greater increase in the venular component.

Our observations on maximal tubular reabsorptive capacity for glucose reveal no significant reduction following L-nor-epinephrine, in contrast to the results of Moyer and Handley[22] who used relatively larger doses in dogs. If T_mG be considered an index of functioning nephrons, our findings do not suggest exclusion of any appreciable number of glomeruli from active circulation, either by vascular shunt or complete ischemia of scattered nephrons. It would appear that the diminished blood flow reflects diffuse involvement of the renal vasculature. T_mG is also unaffected by epinephrine.[24]

Several possible mechanisms for the production of proteinuria following the adrenal medullary amines may be considered. Marked renal ischemia of itself, by causing either alterations in glomerular permeability or diminution in tubular reabsorptive capacity for protein, could produce proteinuria. The latter is less likely since maximal reabsorption and excretory capacities for glucose and p-aminohippurate, respectively, are not affected by these amines. These parameters probably are not reliable indices of tubular capacity for protein reabsorption. Furthermore, although normal filtration and reabsorption of protein by the human kidney is probable, conclusive evidence for this is lacking. Elevated intraglomerular pressure, as evidenced by increased filtration fraction and net efferent resistance, would suggest a direct hydrostatic effect favoring diffusion of protein through the glomerular membrane. However, continuing proteinuria during the recovery periods when the filtration fraction was returning toward normal, makes persistence of the effects of renal ischemia a more likely explanation. At present, precise definition of the mechanisms underlying the

proteinuria induced by the sympathomimetic amines is not possible.

Transient proteinuria is frequently observed in normal persons under stressful conditions, such as cold, fright and severe exercise, all of which are characterized by renal vasoconstriction. Marked reduction in renal blood flow and occurrence of proteinuria has been demonstrated by us in normal subjects developing syncope following prolonged erect immobilization.[8] There are some individuals who demonstrate proteinuria intermittently with relatively minor provocation other than orthostasis. These individuals may be differentiated from patients with fixed postural proteinuria, which appears regularly on assumption of the erect position. Proteinuria in the latter group has been attributed to a variety of organic renal lesions, such as latent glomerulonephritis, pyelonephritis and congenital abnormalities.[3,25] Emotional and vasomotor instability is conspicuous in intermittent proteinuria, with many of the features of "neurocirculatory asthenia."[1,2,4] Abnormal adjustment to postural change or emotional stimuli with marked reduction in renal blood flow may account for proteinuria in such subjects. This response may be mediated either through neurogenic pathways or the adrenal medullary amines.

We found no evidence that subsequent renal damage of any degree resulted from single intravenous infusion of the amines. It is recognized that the medullary hormones may be involved in systemic hemodynamic adjustments, particularly of an emergency nature.[26] The ultimate effect of repeated mobilization of these hormones with attendant renal ischemia is not known. In this connection, severe transient and permanent renal injury may occur in association with pheochromocytoma[27] in which large amounts of these hormones appear recurrently in the circulation.

CONCLUSIONS

1. Intravenous L-nor-epinephrine and epinephrine (U.S.P.) induce proteinuria regularly in man.

2. In dosage of 15 to 44 μg./min. these adrenal medullary amines produce reduction in renal plasma flow, glomerular filtration rate and urinary volume, with an increase in filtration fraction.

3. Over-all increase in renal vascular resistance occurs with participation of all components, preponderantly afferent in the case of L-nor-epinephrine and equally afferent and venular with epinephrine.

4. The maximal tubular reabsorptive capacity for glucose is unaffected by L-nor-epinephrine, indicating that the renal ischemia diffusely involves all nephrons.

5. Demonstration that proteinuria together with renal ischemia is induced by the adrenal medullary amines suggests that these hormones play a role in the transient proteinuria of various stressful conditions.

REFERENCES

1. Christian, H. A. Bright's Disease. New York, 1948. Oxford University Press.
2. Post, W. E. and Thomas, W. A. Orthostatic albuminuria. *J. A. M. A.*, 80: 293, 1923.
3. King, S. E. Patterns of protein excretion by the kidneys. *Ann. Int. Med.*, 42: 296, 1955.
4. King, S. E. Proteinuria variations in the differentation of renal disorders. *J. A. M. A.*, 155: 1023, 1954.
5. Ahrenheim, J. H. Emotional albuminuria in combat. *Psychosom. Med.*, 9: 51, 1947.
6. Chesley, L. C., Markowitz, I. and Wetchler, B. B. Proteinuria following momentary vascular constriction. *J. Clin. Investigation*, 18: 51, 1939.
7. Odell, L. D. and Aragon, G. T. Cold pressor test and kidney function. *Am. J. Obst. & Gynec.*, 54: 870, 1947.
8. King, S. E. and Baldwin, D. S. Unpublished data.
9. Smith, H. W. The Kidney. New York, 1951. Oxford University Press.
10. Starr, I., Jr. The production of albuminuria by renal vasoconstriction in animals and man. *J. Exper. Med.*, 43: 31, 1926.
11. Goldring, W. and Chasis, H. Hypertension and Hypertensive Disease. New York, 1944. The Commonwealth Fund.
12. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B. and Graber, M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Investigation*, 24: 388, 1937.
13. Bodansky, M. and Bodansky, O. Biochemistry of Disease. New York, 1952. The Macmillan Co.
14. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, 153: 375, 1944.
15. Gomez, D. M. Calculation of effective afferent and efferent renal resistance. *Federation Proc.*, 7: 41, 1948.
16. Fowler, N. O., Westcott, R. N., Scott, R. C. and McGuire, J. The effect of norepinephrine upon pulmonary arteriolar resistance in man. *J. Clin. Investigation*, 30: 517, 1951.
17. Iglauer, A. and Altschule, M. D. The effect of paredrine on the venous system. *J. Clin. Investigation*, 19: 503, 1940.
18. Addis, T., Barrett, E., Boyd, R. I. and Ureen, H. J. Renin proteinuria in the rat. *J. Exper. Med.*, 89: 131, 1949.

19. Barnett, A. J., Blacket, R. B., Depoorter, A. E., Sanderson, P. H. and Wilson, G. M. The action of noradrenaline in man and its relation to phaeochromocytoma and hypertension. *Clin. Sc.*, 9: 151, 1950.
20. Smythe, C. M., Nickel, J. F. and Bradley, S. E. The effect of epinephrine (U.S.P.), L-epinephrine and L-norepinephrine on glomerular filtration rate, renal plasma flow and urinary excretion of sodium, potassium and water in normal man. *J. Clin. Investigation*, 31: 499, 1952.
21. Pullman, T. N. and McClure, W. W. The response of the renal circulation in man to constant speed infusion of L-norepinephrine. *Circulation*, 9: 600, 1954.
22. Moyer, J. H. and Handley, C. A. Norepinephrine and epinephrine effect on renal hemodynamics with particular reference to vascular shunting, and decreasing the active glomeruli. *Circulation*, 5: 91, 1952.
23. Nickel, J. F., Smythe, C. M., Papper, E. M. and Bradley, S. E. A study of the mode of action of the adrenal medullary hormones on sodium, potassium and water excretion in man. *J. Clin. Investigation*, 33: 1687, 1954.
24. Smith, H. W., Goldring, W., Chasis, H., Ranges, H. A. and Bradley, S. E. The application of saturation methods to the study of glomerular and tubular function in the human kidney. *J. Mt. Sinai Hosp.*, 10: 59, 1943.
25. Rytand, D. A. The renal lesion in orthostatic albuminuria. *Ann. Int. Med.*, 40: 699, 1937.
26. Von Euler, U. S. Physiologic basis for the clinical use of noradrenalin. *Ann. Int. Med.*, 41: 699, 1954.
27. Fertig, H. H., Taylor, R. D., Corcoran, A. C. and Page, I. H. The renal manifestations of pheochromocytoma. *Ann. Int. Med.*, 35: 1358, 1951.

Metabolic Observations during the Forced Feeding of Patients with Cancer*

A. Raymond Terepka, M.D.† *and* Christine Waterhouse, M.D.

Rochester, New York

The weight loss associated with progressive tumor growth in humans is a well recognized clinical phenomenon. One factor obviously responsible for this is the profound anorexia which almost invariably develops in such patients, although the exact mechanism responsible for this is not known. An additional factor which plays a major role in the development of cachexia appears to be an increase in energy expenditure of the cancer-bearing host so that body fat stores are called upon to make up the deficit between intake and output of energy. This has been shown directly by Mider and his associates in the rat[1,2] and indirectly by Waterhouse, Fenninger and Keutmann in man.[3]

It would be of considerable value, from the clinical point of view, to correct or preserve the body nutrition in terminal cancer patients. To improve body nutrition by correcting the factor of increased caloric expenditure is not possible in most cancerous patients since a remission of the malignant disease must be produced.[3] Direct stimulation of appetite by the production of hypothalamic lesions or the injection of gold-thioglucose has been attempted in animals but obviously does not lend itself to application to humans. Consequently, at the present time the only feasible method available to attempt correction of the large caloric deficit commonly encountered in patients with cancer appeared to us to be by force supplementation of the patients' voluntarily reduced intake.

This article reports our observations on patients with a growing cancer while under such treatment. In order to evaluate fully the metabolic effects of such a forced feeding regimen, complete balance studies were deemed necessary in addition to close clinical observation on the metabolism ward.

PATIENTS AND METHODS

The patients utilized in the study were selected to represent a variety of malignant diseases. The malignant process was widespread and progressive in each and a positive diagnosis had been established at operation or by biopsy. All subjects were admitted to the metabolism ward where well controlled balance studies could be conducted.

Pertinent data referable to the eight patients studied are given in Table 1. Subject A. D. was studied on two occasions with a sixteen-month interval between and these two experiments are designated throughout the remainder of the paper as A. D.#1 and A. D.#2. All of the subjects except A. D. during his first study, appeared chronically ill, had lost a considerable amount of weight and complained of anorexia. Before the start of each experiment the subjects were allowed to choose their own diet. When the voluntary caloric intake was established a single diet or two alternating diets were made up at this caloric level with a normal distribution of protein, carbohydrate and fat and this was eaten during the remainder of the experiment. After suitable control periods the diets were increased in both nitrogen and calories to levels which, when possible, met or exceeded the caloric expenditures during the control periods. This proved to be an increase of from 50 to 100 per cent above the control values. Our aim during the forced feeding periods was to continue to supply a diet relatively normal in its distribution of protein, carbohydrate and fat. In T. M., who was to be given intravenous albumin as part

* From the Department of Medicine, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y. This investigation was supported by research grants from the National Cancer Institute of the National Institutes of Health, United States Public Health Service (C-739[C]), and from the American Cancer Society, Inc. A portion of these data were presented at a meeting of the New England Cancer Society.

† Fellow of the American Cancer Society.

TABLE 1

CLINICAL DATA AND DIETARY INTAKE OF PATIENTS STUDIED

Patient	Sex and Age	Type of Tumor	Total Days Balance	Initial Control Periods					Forced Feeding Periods				
				Dietary Intake				Caloric Output (per day)	Dietary Intake				Days
				Nitrogen*	Carbohydrate*	Fat*	Calories†		Calories†	Nitrogen*	Carbohydrate*	Fat*	
E. C.	M, 63	Carcinoma prostate	39	10.08	157	98	1,762	2,092	3,095	17.80	317	150	9
									4,702	27.57	434	248	10
A. D.#1	M, 59	Malignant melanoma	28	10.02	261	80	2,014	4,774	3,074	15.45	292	170	8
A. D.#2	M, 60	Malignant melanoma	42	7.92	171	66	1,532	4,171	2,577	12.81	248	166	12
I. E.	F, 44	Carcinoma bladder	46	6.10	88	56	1,013	1,694	2,677	12.17	168	190	16
S. H.	F, 50	Sarcoma uterus	24	6.52	167	76	1,515	3,379	3,224	15.13	350	167	12
H. K.	F, 31	Monocytic leukemia	63	8.45	161	70	1,485	1,882	2,258	12.57	232	222	6
T. M.	M, 60	Carcinoma pancreas	24	10.81	176	102	1,850	3,142	3,105	.....	341	238	12
A. M.	F, 61	Lympho-sarcoma	26	8.46	151	84	1,559	3,888	3,180	12.83	214	226	8
C. W.	M, 68	Carcinoma bronchus	24	7.40	163	74	1,449	2,510	2,675	14.60	305	134	8

* Grams per day.
† Calories per day.

of another study, the dietary nitrogen was not increased during forced feeding. Supplementation was accomplished by the use of concentrated oral feedings* given, when necessary, by means of intubation. It soon became obvious that these large involuntary increases in food intake were not well tolerated over long periods of time, even when the feedings were given by gastric tube, and for this reason the periods of forced feeding were relatively short in some cases. The patients frequently complained of uncomfortable fullness and occasional nausea after a feeding but vomiting was rare. In two cases (T. M. and S. H.) diarrhea of moderate severity developed while on the forced feeding diet.

All diets were planned, weighed and cooked on the metabolism ward, under the direct supervision of the investigators, by a specially trained dietitian and nursing staff. Any refused food or emesis was saved for analysis. Urine was collected in periods of twenty-four hours and stools were saved and pooled into four to eight day periods after separation by carmine markers.

* Somagen® (Upjohn) was used as the principal source of nitrogen, dexin® (Burroughs Wellcome) for carbohydrate and lipomul-oral® (Upjohn) for calories. In A. D.#2 and C. W., Ediol® (Schenley) was used as the caloric supplement. We are indebted to Dr. Robert W. Heinle of the Upjohn Company for a generous supply of lipomul-oral.

Measured amounts of distilled water were given ad libitum.

Balances of nitrogen, phosphorus, calcium, potassium, sodium and chloride were determined throughout the experiments in all patients. In T. M., for reasons already stated, only balances during the initial periods and chloride balances during the forced feeding periods are pertinent to this study. The intakes of these elements were determined at least in duplicate by analyses of sample diets identical in all respects to the ones eaten by the patients. The analytic procedures used for the diets, urine and stools were similar to those outlined in previous publications.[4] The caloric values and the proportions of carbohydrate and fat in the diets were calculated from tables. The composition of various diets used is given in Table 1.

Throughout the experiment repeated determinations of the basal metabolic rate were made in the patients, using a standard closed circuit Benedict-Roth apparatus. Nude body weights were obtained daily to an accuracy of 10 gm. and caloric expenditures were calculated for each period using the method outlined by Newburgh.[5] The technic depends on accurate determination of insensible weight loss and from this a subsequent calculation of the amount of heat dissipated. Calculations of caloric expendi-

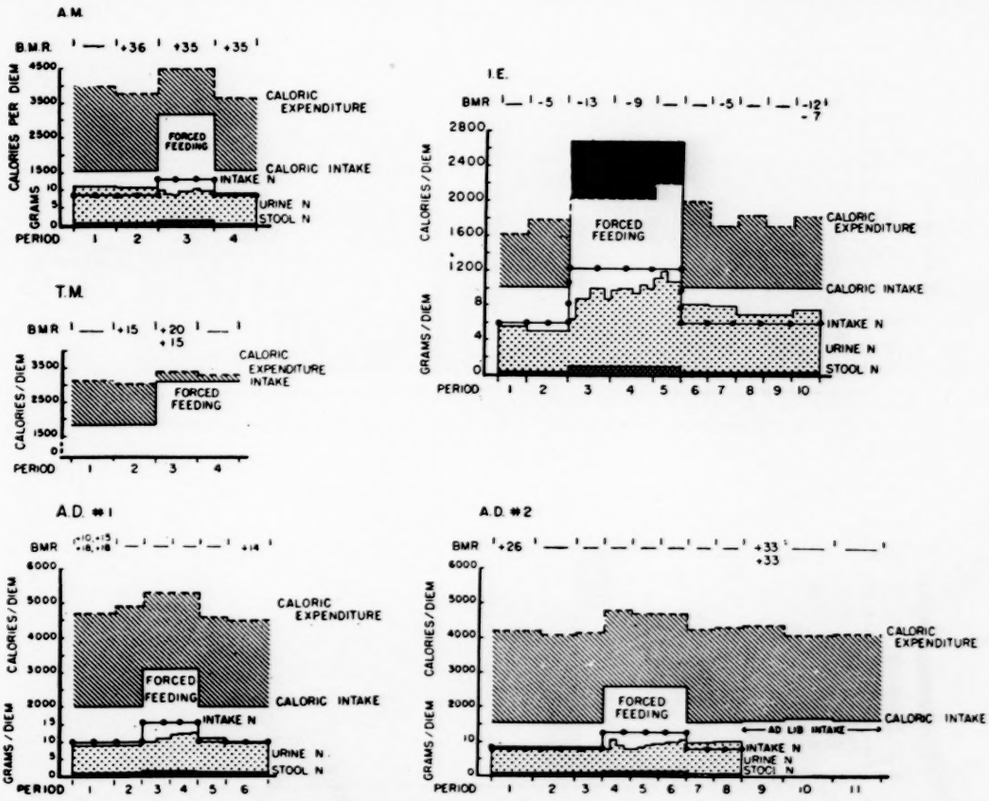


FIG. 1. Caloric balances, nitrogen balances and basal metabolic rates before, during and after forced feeding of patients with cancer. Negative caloric balances are indicated by the diagonally hatched areas while positive caloric balances are indicated by the darker areas with the white dots. The ordinates for both caloric and nitrogen balances have the same base lines. Note that the caloric expenditures and basal metabolic rates in these patients did not change significantly after forced feeding.

tures by the modification suggested by Lavietes[6] from the quantities of nitrogen, carbohydrate and fat metabolized, yielded results almost identical with those obtained by using the factor 2.26 suggested by Newburgh.[7] Therefore, this latter method was used in most of the later experiments.

Calculations of body compartments and intracellular shifts of sodium were carried out using the changes in chloride balance, after correction for skin loss, to indicate extracellular fluid volume, based on the procedure suggested by Lavietes, D'Esopo and Harrison.[8]

RESULTS

Caloric Expenditures and Basal Metabolic Rates. It is readily apparent from examination of Figures 1, 2 and 5 that from the point of view of changes in caloric balance and basal metabolic rate determinations, the patients may be divided into two groups, one represented by A. M., I. E., A. D.#1, A. D.#2 and probably T. M., and the other by S. H., H. K., C. W. and E. C. In the former group (Fig. 1) the caloric expenditures increased only slightly during forced feeding (? increased specific dynamic action) and the caloric balances generally became much less negative or slightly positive. There were no significant changes in the basal oxygen consumption and the caloric expenditures returned promptly to control levels after supplementation was discontinued. In the latter group of patients (Figs. 2 and 5) marked increases in the basal metabolic rates and caloric expenditures were noted during or immediately following supplementation. E. C. was the only patient who could be studied for a long period of time after forced feeding since the three other patients died very soon after termination of the experiments. This will be discussed more fully along with the clinical response of these patients. In E. C. what may be considered a long-term

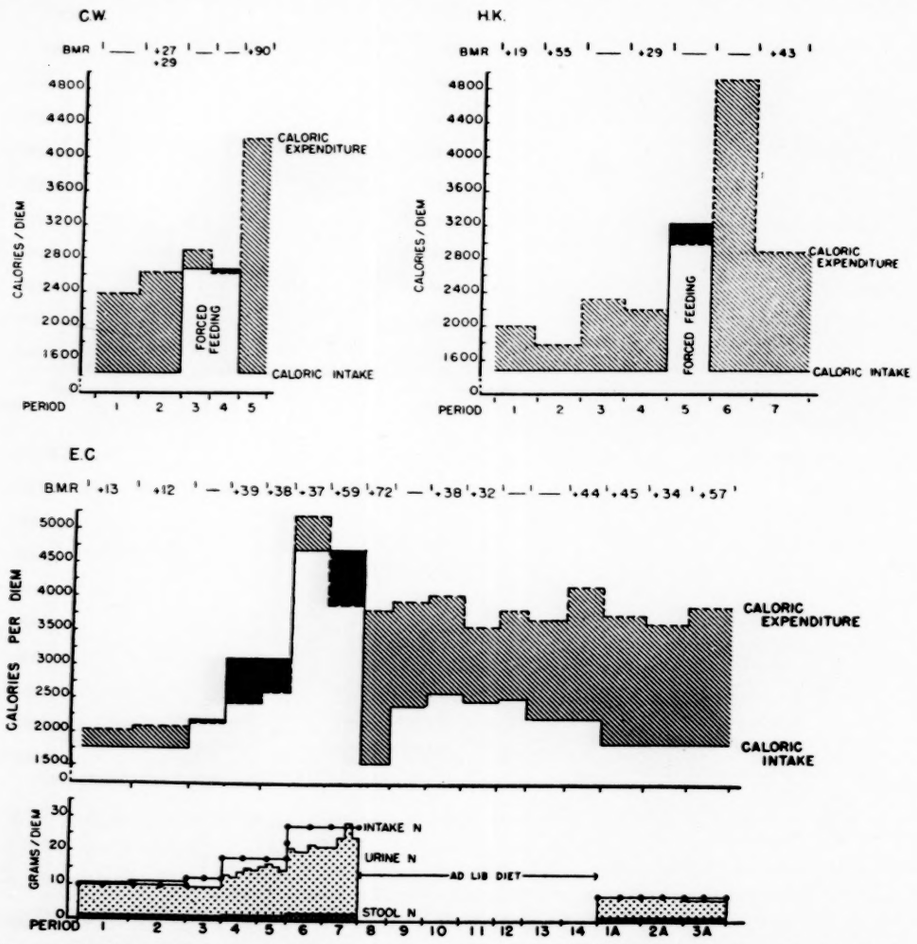


FIG. 2. Caloric balances, nitrogen balances and basal metabolic rates before, during and after forced feeding of patients with cancer. Negative caloric balances are indicated by the diagonally hatched areas while positive balances are indicated by the darker areas with the white dots. Note that the caloric expenditures and basal metabolic rates in these patients were significantly higher after forced feeding.

deleterious effect of forced supplementation may be seen. Once the increase of caloric expenditure had occurred, this patient apparently was unable to regain his former level in spite of the subsequent reduced caloric intake.

It should be noted that in two instances (E. C. and I. E.) the calculated caloric expenditures during the control periods were in what would be considered a normal range. However, because of low intake, a caloric deficit was still present, suggesting again that cancer patients may be unable to react normally to restriction of caloric intake.[9]

Body Weight. Gross body weights were decreasing or remained about steady in seven of the nine experiments during the initial control periods while in A. D.#1 and S. H. substantial gains in body weight occurred during this time. (Table II and Figs. 3, 4 and 5.) During forced feeding body weights increased in all but these same two patients. These increases were often substantial and gains up to 390 gm. per day were recorded. The average for the group was 240 gm. per day. It is to be noted, however, that on discontinuing supplementation body weights uniformly decreased and, more significantly, at a greater rate than that which might have been occurring previous to forced feeding.

The reasons that patients A. D.#1 and S. H. gained weight during the initial control periods and failed to do so during the time of forced feeding will be apparent from the discussion in the section on fluid and electrolyte shifts to follow. It again emphasizes the fact that inter-

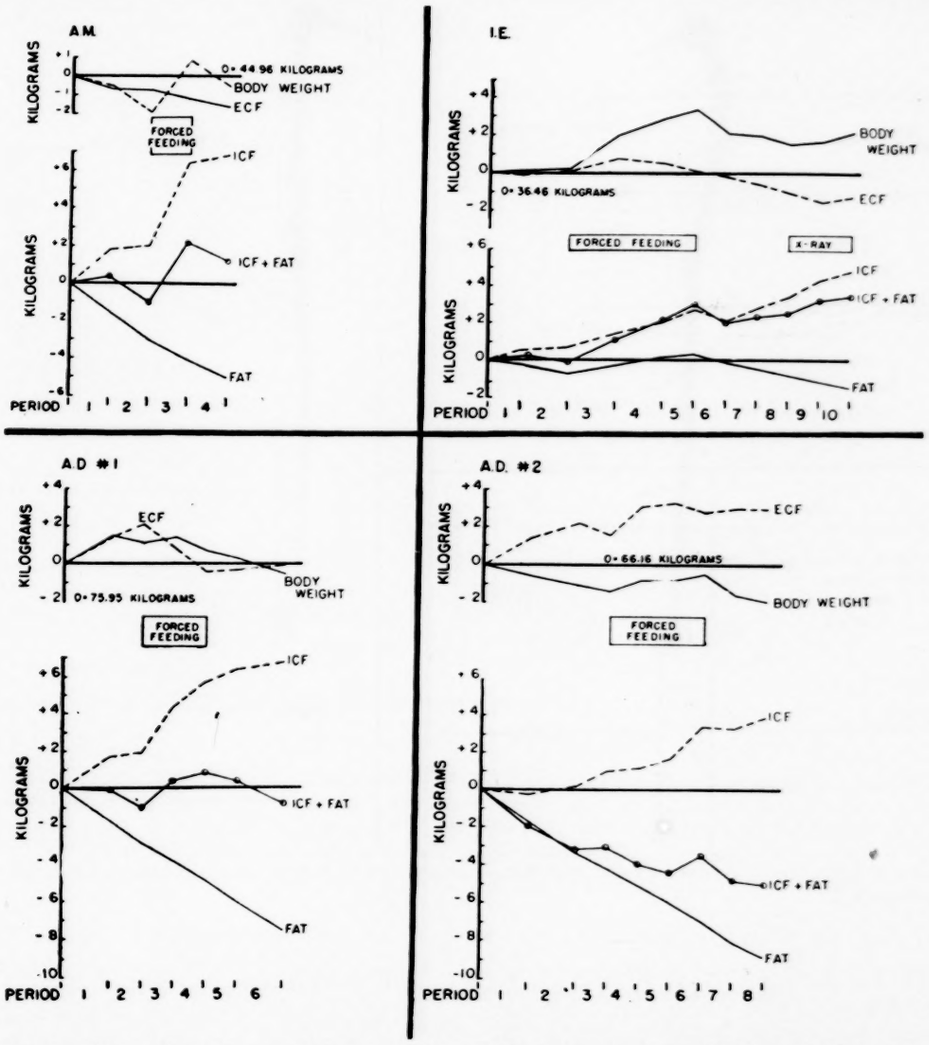


FIG. 3. Cumulative changes in body weight and body compartments before, during and after the forced feeding of patients in Figure 1. See text for explanation of symbols.

pretation of weight changes, especially in cancer patients, is hazardous without knowledge of the simultaneous electrolyte changes.[3]

Balance Studies. Nitrogen balance data for the patients studied are shown in Table II and in Figures 1, 2 and 5. During control periods a significant deviation from balance was noted only in A. M. She lost an average of 2.6 gm. of nitrogen per day. With the institution of forced feeding a significant positive nitrogen balance was established in all patients. It should be noted, however, that as the increased nitrogen intake was maintained there appeared to be a gradual return toward equilibrium. In A. M., who was in rather marked negative balance to begin with, this was not as readily apparent (Fig. 1, Table II) and in H. K. nitrogen balance data were complicated by the administration of blood transfusions which were necessary during periods III and VI. (Table II.) Nevertheless, the nitrogen balances became negative throughout the postsupplementation control periods in all patients. S. H. died on the day following the last forced feeding period, and T. M. had no post-supplementation control periods for reasons previously mentioned.

It is not surprising, from consideration of the nitrogen balance data, that all patients except S. H. retained more phosphorus during the periods of forced feeding than during the control periods. An unexpected finding was the occurrence of a strongly negative calcium balance in

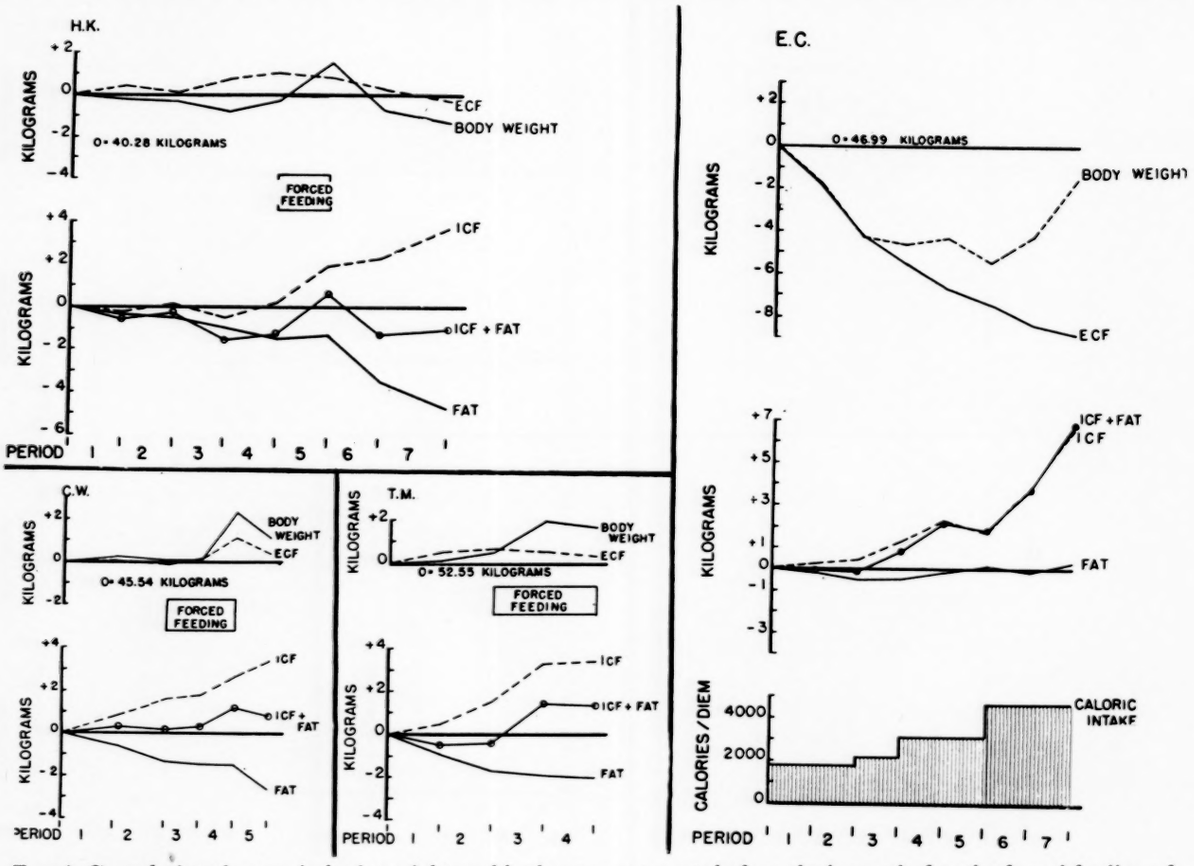


FIG. 4. Cumulative changes in body weight and body compartments before, during and after the forced feeding of patients in Figure 2.

four cases (I. E., A. D.#1, A. D.#2 and H. K.) while both nitrogen and phosphorus balances were positive. This observation, however, correlates well with the fact that the amount of phosphorus retained from the diet in these four patients was uniformly less than that retained by those patients in whom the calcium balance remained positive. Therefore, when the observed balances were corrected for calcium excretion, all patients retained phosphorus in roughly the same order of magnitude. (Table II.) These findings suggest that during the period of supplementation, with retention of nitrogen, the demand for phosphorus may have been great enough to lead to withdrawal of additional phosphorus from bone in order to meet this demand. In S. H. failure to increase phosphorus retention correlates with her failure to retain significant amounts of nitrogen during supplementation.

Whereas theoretic nitrogen balances from phosphorus in all these patients agreed fairly well during the initial control periods, during supplementation rather large discrepancies usually occurred in that more phosphorus was retained than could be accounted for by the nitrogen and calcium balances. (Table II.) This possibly is a reflection of the altered phosphorus to nitrogen ratio in tumor tissues which has been previously described.[4]

Again with the exception of S. H. all the patients retained more potassium during the forced feeding period than that retained during the pre-or postsupplementation control periods. This also suggests that increased protein anabolism was occurring during this time.

Fluid and Electrolyte Shifts. Changes in extracellular fluid volume were neither striking nor completely uniform. E. C., who was clinically edematous at the start of the experiment, lost extracellular fluid continuously. The rest of the patients, with the exception of A. D. #1 and S. H., either had slight to moderate gains in extracellular fluid or this remained constant. (Figs.

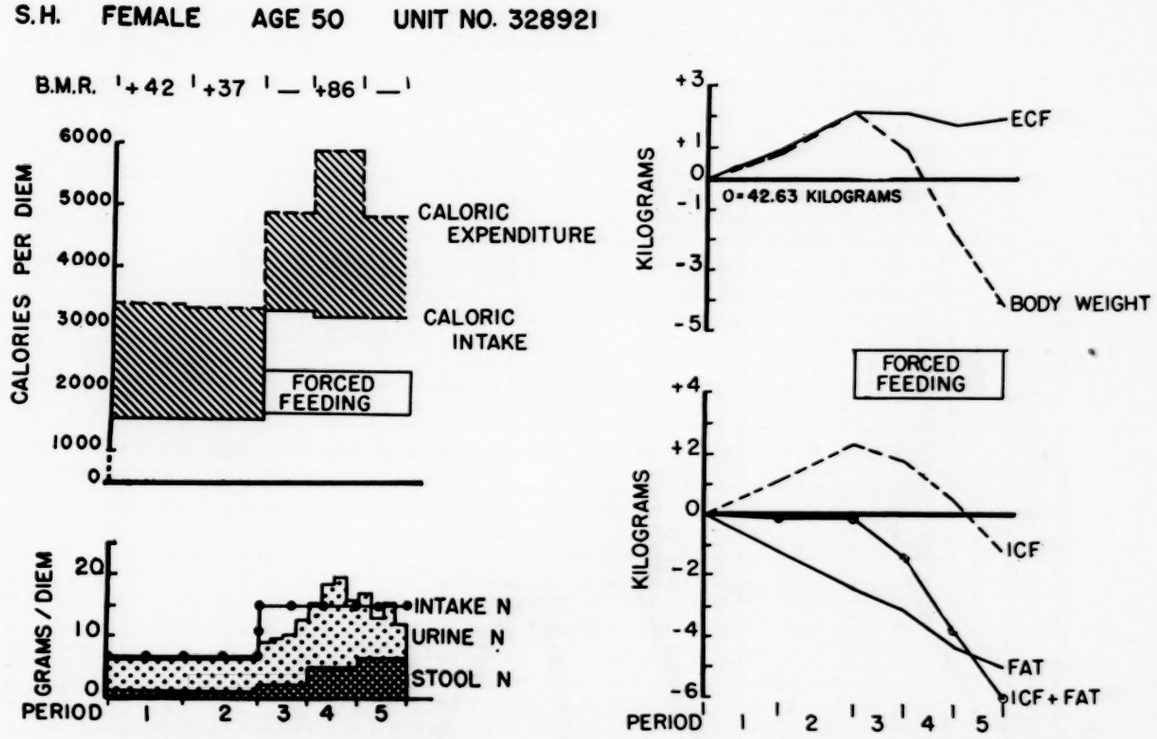


FIG. 5. Caloric balances, nitrogen balances, basal metabolic rates and cumulative body weight, and body compartment changes in a patient with sarcoma of the uterus before and during forced feeding. The patient died on the day following termination of the last forced-feeding period. Note the marked elevation in caloric expenditure and basal metabolic rates, and the rapid weight loss during forced feeding.

3, 4 and 5.) The substantial gains of extracellular fluid in A. D.#1 and S. H. can be seen to account for all of the weight gain that occurred in these two patients throughout the initial control periods.

During and after forced feeding the changes in extracellular fluid were usually small and variable, again excepting A. D.#1. During his period of supplementation the extracellular fluid which had been gained previously was mobilized and excreted rapidly so that a net loss of body weight occurred. Previous to the start of the experiment this patient had received large quantities of albumin intravenously and his intake of sodium during the control period was markedly increased over that given previously.

Total sodium balances throughout all experiments followed closely those that occurred in the chloride balances from which the extracellular fluid changes described were calculated.

The discrepancy between the observed changes in body weight and the calculated changes in extracellular fluid and protein (the latter from the nitrogen balance) is considered to be the contribution of fat and intracellular fluid to alterations of the body weight of an individual. As will be seen in Figures 3, 4 and 5 the weight gained or lost by our patients during and after forced feeding was due largely to changes in intracellular fluid plus fat.

It would be very interesting to verify the actual magnitude of the contribution of each of these components to the observed changes in body weight. It has been stated by Brozek and Keys[10] that in the early stages of gaining weight after severe undernutrition, about one-half of the weight gained is due to accumulation of fat and this becomes proportionately greater during later stages of repletion. Our data do not warrant any direct analysis of these changes but if one assumes that the calculated caloric discrepancy is approximately correct and that this is all made up by body fat stores, in every instance a gain in weight as a result of forced feeding was due almost entirely to a gain in intracellular fluid.

The potassium balances support this conclusion. Using these balances and the various

TABLE II

METABOLIC BALANCE DATA FROM PATIENTS STUDIED

(The Forced Feeding Periods are Enclosed by the Broken Lines)

PATIENT	PERIOD	NO. OF DAYS	BODY WEIGHT[3]	NITROGEN[1]				THEORETICAL NITROGEN FROM PHOSPHORUS	PHOSPHORUS[1]				PHOSPHORUS BALANCE CORRECTED FOR CALCIUM[1]	CALCIUM[1]				POTASSIUM[2]				SODIUM[2]					CHLORIDE[2]				
				INTAKE	URINE	STOOL	BAL.		INTAKE	URINE	STOOL	BAL.		INTAKE	URINE	STOOL	BAL.	INTAKE	URINE	STOOL	BAL.	INTAKE	URINE	STOOL	BAL.	SERUM CONC.[4]	INTAKE	URINE	STOOL	BAL.	SERUM CONC.
AD#1	I	6	75.95	10.02	7.91	.93	+1.18	+ .4	1.11	.755	.33	+.02	-.03	.61	.141	.47	0	73	65	2	+ 6	95	63	0	+32	141	90	63		+27	104
	II	4	77.46 77.09	10.02	8.09	.93	+1.00	- .1	1.11	.782	.33	0	-.01	.61	.127	.47	+.01	73	59	2	+12	95	75	0	+20	138 136	90	70		+20	101 99
	III	4	77.31	15.45	8.89	1.59	+4.97	+3.7	1.56	.793	.59	+.18	+.25	1.10	.168	1.10	-.17	87	61	4	+22	98	103	1	- 6	138	100	105		- 5	102
	IV	4	76.64	15.45	10.57	1.59	+3.29	+1.9	1.56	.912	.59	+.06	+.13	1.10	.173	1.10	-.17	87	68	4	+15	98	96	1	+ 1	139	100	97		+ 3	107
	V	4	76.27	10.02	9.88	1.35	- 1.21	- 1.3	1.11	.838	.44	-.17	-.09	.61	.137	.64	-.17	73	70	4	- 1	95	92	1	+ 2	137	90	88		+ 2	104
	VI	6	75.37	10.02	8.48	1.35	+ .19	+ .4	1.11	.720	.44	-.05	+.03	.61	.131	.64	-.16	73	63	4	+ 6	95	89	1	+ 5	139	90	80		+10	103
AD#2	I	6	75.99	7.92	7.76	.81	- .65	- 1.6	1.00	.736	.40	-.14	-.11	.74	.207	.58	-.05	58	50	2	+ 6	87	65	1	+21	142	86	62		+24	106
	II	6	76.92 76.57	7.92	7.82	.81	- .71	- .7	1.00	.698	.40	-.10	-.05	.74	.241	.58	-.08	58	45	2	+11	87	74	1	+12	138 137	86	70		+16	99 95
	III	4	75.95	7.92	7.62	.81	- .51	- 1.2	1.00	.709	.40	-.11	-.08	.74	.206	.58	-.05	58	51	2	+ 5	87	78	1	+ 8	142	86	72		+14	97
	IV†‡	4	77.46	12.61	7.34	1.94	+3.33	+4.1	1.49	.510	.89	+.09	+.28	1.55	.220	1.76	-.43	74	42	13	+19	120	73	9	+38	137	126	77	4	+45	96
	V	4	77.09	12.81	7.38	1.21	+4.22	+5.3	1.51	.730	.56	+.22	+.36	1.57	.339	1.53	-.30	76	50	3	+23	122	122	1	- 1	136	132	117		+15	94
	VI†	4	77.31	12.66	8.33	.80	+3.53	+2.6	1.49	.926	.40	+.16	+.18	1.54	.336	1.22	-.02	75	54	4	+17	121	94	1	+26	134	128	93		+35	101
	VII	4	76.64	7.92	9.23	.64	- 1.95	+ .6	1.00	.765	.34	-.10	+.04	.74	.287	.77	-.32	58	56	1	+ 1	87	78	0	+ 9	138	86	79		+ 7	99
	VIII	4	76.27	7.92	9.53	.64	- 2.25	+1.5	1.00	.704	.34	-.04	+.10	.74	.278	.77	-.31	58	53	1	+ 4	87	68	0	+19	134	86	66		+20	100
I.E.	I	4	36.46	6.10	5.30	.33	+ .47	+1.9	.85	.326	.24	+.28	+.13	.85	.093	.41	+.35	45	46	1	- 2	51	46	0	+ 5	143	51	47		+ 4	110
	II	6	36.53 36.65	6.10	4.69	.33	+1.08	+2.5	.85	.302	.24	+.31	+.17	.85	.126	.41	+.32	45	41	1	+ 3	51	34	0	+17	140 149	51	35		+16	109 108
	III	6	38.49	12.17	7.72	1.05	+3.40	+8.4	1.63	.429	.86	+.34	+.57	1.50	.233	1.78	-.51	69	49	5	+15	64	47	1	+16	138	72	56		+16	105
	IV	6	39.36	12.17	8.88	1.05	+2.24	+7.5	1.63	.504	.86	+.27	+.51	1.50	.267	1.78	-.55	69	61	5	+ 3	64	55	1	+ 8	141	72	69		+ 3	104
	V	4	39.86	12.17	10.09	1.05	+1.03	+9.6	1.63	.360	.86	+.41	+.65	1.50	.254	1.78	-.53	69	65	5	- 1	64	60	1	+ 3	145	72	71		+ 1	106
	VI	4	38.59	6.10	7.72	.43	- 2.05	- 1.2	.85	.539	.38	-.07	-.08	.85	.169	.64	+.04	45	50	1	- 6	51	51	1	- 1	140	51	57		- 6	101
	VII	4	38.41	6.10	7.54	.43	- 1.87	+1.2	.85	.381	.38	+.09	+.08	.85	.179	.64	+.03	45	46	1	- 2	51	48	0	+ 3	142	51	48		+ 3	104
	VIII	4	38.10	6.10	6.47	.43	- .80	+1.3	.85	.363	.38	+.11	+.09	.85	.153	.64	+.06	45	43	1	+ 1	51	47	0	+ 4	143	51	53		- 2	105
	IX*	4	38.18	6.10	6.59	.43	- .92	0	.85	.422	.38	+.05	.00	.85	.100	.64	+.11	45	40	1	+ 4	51	44	0	+ 7	146	51	55		- 4	106
	X*	4	38.78	6.10	7.13	.43	- 1.46	+ .3	.85	.379	.38	+.09	+.02	.85	.030	.64	+.18	45	44	1	0	51	30	0	+21	136	51	37		+14	102
T.M.	I	6	52.55	10.81	8.63	1.40	+ .78	+1.8	1.38	.578	.75	+.05	+.12	1.12	.094	1.18	-.15	72	61	6	+ 5	64	51	1	+12	137	76	63		+13	101
	II	6	52.61 53.04	10.81	8.85	1.40	+ .56	+1.2	1.38	.630	.75	0	+.08	1.12	.113	1.18	-.17	72	61	6	+ 5	64	56	1	+ 7	138 139	76	73		+ 3	101 99
	III	6	54.55																							35	29		+ 6	101
	IV	6	54.25																							35	32		+ 3	103
A.M.	I	6	44.96	7.98	10.08	.86	- 2.96	- 4.8	1.06	.727	.59	-.26	-.33	.84	.048	.64	+.15	56	49	10	- 3	70	62	4	+ 4	136	61	65		+ 6	101
	II	6	44.42 43.01	8.46	9.88	.86	- 2.28	- 4.1	1.07	.684	.59	-.20	-.28	.84	.058	.64	+.14	57	46	10	+ 1	71	57	4	+10	136 138	62	53		+ 9	104 102
	III	8	45.91	12.83	8.26	1.17	+3.40	+4.8	1.57	.709	.38	+.48	+.33	1.22	.216	.66	+.34	69	30	12	+27	75	47	5	+23	137	68	54		+14	109
	IV	6	44.48	8.46	8.77	.53	- .84	- 2.1	1.07	.723	.37	-.02	-.14	.84	.234	.34	+.27	57	54	4	- 1	71	82	1	-12	136	62	64		- 2	104

TABLE II (*Continued*)

PATIENT	PERIOD	NO. OF DAYS	BODY[3] WEIGHT	NITROGEN[1]				THEORETICAL NITROGEN FROM PHOSPHORUS	PHOSPHORUS[1]				PHOSPHORUS BALANCE CORRECTED FOR CALCIUM[1]	CALCIUM[1]				POTASSIUM[2]				SODIUM[2]					CHLORIDE[2]				
				INTAKE	URINE	STOOL	BAL.		INTAKE	URINE	STOOL	BAL.		INTAKE	URINE	STOOL	BAL.	INTAKE	URINE	STOOL	BAL.	INTAKE	URINE	STOOL	BAL.	SERUM CONC.[4]	INTAKE	URINE	STOOL[5]	BAL.	SERUM CONC.[4]
			46.99																						130					100
	I	7	45.20	10.08	9.41	.91	-0.24	+ 1.2	1.34	.604	.60	+ .14	+ .08	.98	.096	.79	+.09	72	58	9	+ 4	45	54	1	- 10	134	52	60		- 8	105
	II	8	42.62	10.08	10.22	.91	- 1.05	+ .6	1.34	.673	.60	+ .07	+ .04	.98	.130	.79	+.06	72	65	9	- 2	45	82	1	-38	134	52	81		-29	100
	III	5	42.33	11.95	8.73	.78	+2.44	+ 2.1	1.07	.354	.47	+ .25	+ .14	1.21	.206	.77	+.23	59	36	8	+15	6	26	1	-21	136	26	42		-16	100
	IV	5	42.64	17.80	12.52	1.24	+4.04	+ 4.1	1.47	.384	.70	+ .39	+ .28	1.78	.259	1.27	+.25	79	50	15	+14	7	24	1	-18	134	38	51		-13	101
E.C.	V	4	41.54	17.80	14.29	1.24	+2.27	+ 2.6	1.47	.464	.70	+ .31	+ .18	1.78	.215	1.27	+.29	79	49	15	+15	7	11	1	- 5	140	38	43		- 5	104
	VI	5	42.72	27.57	18.86	1.61	15.16	+15.0	2.81	.675	.94	+1.19	+1.02	2.75	.333	2.03	+.39	135	80	17	+38	11	61	1	-51	138	89	98		- 9	104
	VII	5	45.44	27.57	21.77	1.61	+4.19	+ 9.6	2.81	1.095	.94	+ .77	+ .65	2.75	.458	2.03	+.26	135	109	17	+ 9	11	41	1	-31	133	89	90		- 1	99
	IA	6	46.11	7.53	6.42	1.03	+ .08	+ 1.6	1.04	.629	.35	+ .06	+ .11	.70	.240	.56	-.10	58	41	6	+11	96	60	1	+35	136	86	63		+23	101
			47.07																						133					103
	IIA	6	48.39	7.53	6.26	1.03	+ .24	+ 1.6	1.04	.632	.35	+ .06	+ .11	.70	.251	.56	-.11	58	44	6	+ 8	96	40	1	+55	140	86	45		+41	103
	IIIA	6	49.01	7.53	5.38	1.03	+1.12	+ 1.6	1.04	.541	.35	+ .15	+ .11	.70	.227	.56	-.09	58	41	6	+11	96	63	1	+32	138	86	63		+23	103
	I	6	42.63	6.52	5.24	.90	+ .38	+ 2.5	.94	.374	.42	+ .15	+ .16	.68	.059	.67	-.05	55	32	11	+12	74	37	3	+34	134	78	53		+25	100
			43.45																						130					99
	II	6	44.69	6.52	5.25	.90	+ .37	+ 2.9	.94	.328	.42	+ .19	+ .20	.68	.039	.67	-.03	55	36	11	+ 8	74	35	3	+36	131	78	49		+29	98
S.H.	III†	4	43.56	15.13	8.18	2.14	+4.81	+ 2.4	1.56	.404	.96	+ .18	+ .16	1.59	.025	1.53	+.04	68	44	28	- 4	63	28	11	+24	141	89	61		+28	104
	IV†	4	40.70	15.13	12.56	4.86	-2.29	+ 2.4	1.52	.148	1.12	+ .25	+ .16	1.58	.025	1.36	+.20	68	13	60	- 5	63	4	55	+ 4	148	89	62	5	+22	115
	V†**	4	38.43	14.74	7.87	6.44	+ .43	- 2.1	1.51	.346	1.20	- .04	- .14	1.59	.010	1.59	+.01	66	11	52	+ 3	61	5	53	+ 3	162	87	42	5	+40	123
	I	6	40.28	8.45	7.82	1.23	- .60	+ .7	1.14	.512	.63	0	+ .05	.86	.017	.96	-.12	64	52	10	+ 2	76	63	2	+11	139	76	63		+13	102
			40.08																						137					97
	II	6	39.94	8.45	8.01	1.23	- .79	+ .9	1.14	.510	.63	0	+ .06	.86	.033	.96	-.13	64	49	10	+ 5	76	68	2	+ 6	135	76	61		+15	101
	III▲	6	39.46	10.75	7.36	1.23	+2.16	+ 3.4	1.18	.375	.63	+ .17	+ .23	.86	.031	.96	-.14	68	58	10	0	82	68	2	+12	135	86	73		+13	93
H.K.	IV	6	40.02	8.45	8.56	1.23	- 1.34	+ 1.2	1.14	.485	.63	+ .03	+ .08	.86	.024	.96	-.13	64	53	10	+ 1	76	51	2	+23	139	75	51		+24	96
	V	6	41.80	12.57	7.89	1.43	+3.25	+ 7.6	1.59	.286	.89	+ .41	+ .52	1.24	.039	1.42	-.24	76	50	12	+14	85	61	3	+21	136	90	71	2	+17	101
	VI▲	6	39.54	13.77	9.32	1.31	+3.14	+ 4.4	1.22	.396	.61	+ .21	+ .30	.86	.080	.96	-.18	72	55	4	+13	90	94	2	- 6	139	98	90		+ 8	103
	VII	8	38.97	8.45	10.68	1.31	-3.54	+ .1	1.21	.682	.61	- .08	+ .01	.92	.114	.96	-.15	73	73	4	- 4	62	66	2	- 6	133	61	73		-12	89
	I	6	45.54	7.40	6.68	.51	+ .21	+ 2.9	1.15	.178	.88	+ .09	+ .20	1.43	.107	1.55	-.23	40	32	3	+ 5	23	12	1	+10	133	26	16	2	+ 8	97
			45.77																						133					96
	II	6	45.66	7.40	6.74	.51	+ .15	+ 1.2	1.15	.304	.88	- .03	+ .08	1.43	.126	1.55	-.25	40	36	3	+ 1	23	10	1	+12	139	26	13	2	+11	97
C.W.	III	4	45.36	14.60	10.54	.69	+3.37	+ 7.4	2.28	.243	1.43	+ .61	+ .50	2.85	.083	2.54	+.23	79	47	11	+21	45	14	1	+30	146	52	28	2	+22	104
	IV	4	47.58	14.60	14.10	.69	- .19	+ 3.8	2.28	.484	1.43	+ .37	+ .26	2.85	.082	2.54	+.23	79	67	11	+ 1	45	22	1	+22	133	52	42	2	+ 8	91
	V†	4	46.34	7.24	10.92	.75	- 4.43	- 4.7	1.13	.702	1.07	- .64	- .32	1.40	.110	2.02	-.73	39	39	11	-11	20	22	1	- 3	126	24	13	2	-11	90

[1] Balance values in gm. per day.
[2] Balance values in mEq. per day.
[3] Body weight in kg. at end of period except in first period when both initial and final weights are given.
[4] Serum concentrations in mEq./L. at end of period except in first period when both initial and final values are given.
[5] Usually only one representative stool analyzed for chloride. When no value given, concentration was insignificant.
† Small differences in intake due to emesis.
‡ Diarrhea present at some time during this period.
* Patient received x-ray treatment during this period.
▲ Patient received blood transfusion during this period. ** A rapid rise in blood urea nitrogen occurred during this period.

factors given by Reifenstein, Albright and Wells[11] it can be shown that uniformly more intracellular fluid was accumulated during the weight gain associated with forced feeding than could be accounted for by the balance of nitrogen. If these calculations are valid, this can mean either that during forced feeding intracellular edema developed or that a protein was being accumulated with an abnormal K/N ratio. The very rapid weight loss after forced feeding was discontinued in those patients who gained substantial amounts of body weight is in keeping with the former concept. However, it is also well established that the K/N ratio in tumor patients is abnormally high,[4] so that accumulation of tumor protoplasm during the forced feeding could also contribute to this phenomenon.

It should be emphasized that in all these calculations various assumptions are made which are open to question and therefore the conclusions derived from them can only be tentative. Interestingly, it has been shown in rats[12,13] that one characteristic alteration associated with progressive growth of Walker carcinoma 256 is a steady accumulation of water in both tumor and host. The high total water content of human tumors is also well known.

The response of patient S. H. to forced feeding was entirely different and is shown in Figure 5. It can be seen that she lost over 6 kg. in body weight during a twelve-day forced feeding period while on an intake of about 3,500 calories per day. The major portion of this weight change, as in the other patients studied, was due to a change in intracellular fluid volume. It will be recalled that this patient and A. D.#1 were the only patients studied who failed to gain weight while being force-fed. In A.D.#1 this failure to gain weight was a result of the mobilization and excretion of a large quantity of extracellular fluid which had accumulated during the control periods. An explanation for the marked loss of intracellular fluid and consequently of body weight in S. H. is not as readily apparent. This patient had a very rapid downhill clinical course and died on the day following the last force-feeding period. Her unusual response to forced feeding will be discussed further in the section on clinical response of the patients and in the discussion.

Clinical Response. In all patients except A. D. (on both occasions) and A. M. the caloric expenditure calculated during the control periods was met or exceeded by the forced feeding diet. In spite of this the clinical response of the patients did not appear uniform and was not related to whether or not their caloric expenditures had been met. Three of the patients (A. M., I. E. and E. C.) said they felt somewhat better during the supplementation, two noted no change (T. M., A. D. on both occasions) and three patients seemed to accelerate their downhill course (S. H., C. W. and H. K.) coincident with the institution of forced feeding.

In the three patients who appeared to benefit transiently from the procedure, only in E. C. was it possible to obtain some objective evidence to substantiate this. While on the *ad libitum* diet which followed the period of forced feeding, for a short time he voluntarily ingested more calories than he had in the control period before forced feeding. (Fig. 2.) A. M. and I. E. were very encouraged when they realized they were gaining weight on the new regimen. However, with discontinuance of forced feeding they expressed no desire to ingest more food than that given during the control periods, and in A. D.#2, when his *ad libitum* intake was measured (Fig. 1), this also was true.

The three patients who, from clinical impression, appeared to accelerate their malignant process during forced feeding were among the four patients (S. H., C. W., H. K. and E. C.) in the study who did not lower their caloric expenditures to the control level after discontinuance of the supplementation. (Figs. 2 and 5.) It is perhaps significant that these three patients (S. H., C. W. and H. K.) died from their malignant process, one, sixteen and twenty-one days, respectively, after forced feeding was discontinued. In the other five patients the time of death ranged from fifty-four to 517 days. The patient E. C. seemed to have a paradoxic response in that he also failed to lower his caloric expenditure and basal metabolic rate after forced feeding but did have some return of appetite and a transient feeling of well-being. As noted previously, this patient had a slowly progressing carcinoma of the prostate with the longest and most benign course of the patients studied. What effect this may have had in conditioning the response is not known. It is of interest to note, also, that patient S. H. who exhibited the most fulminating course during feeding had a very rapidly progressing sarcoma and was among the most malnourished of the patients studied.

DISCUSSION

The obvious similarity in the clinical appearance of a terminal cancer patient and a chronically starved individual immediately suggests that nutritional rehabilitation should be attempted. If the malnutrition seen with advancing malignancies is simply a depletion of host tissues secondary to a markedly restricted diet as a result of anorexia and the demands of the growing tumor, the repletion process in cancer patients should be relatively simple. That this would be of profound benefit to the patient is apparent from the fact that many malignancies cause the death of their hosts by some means other than by interference with the proper function of a vital organ, or by the hypersecretion of some biologically active hormone, or by uncontrolled hemorrhage or infection from an ulcerated lesion.[9] Such complications of the basic malignant process are now becoming less frequent, and we are seeing more patients in the hospital with widespread metastases, rapid weight loss and the clinical appearance of death due to starvation.

The possibility that the death of a tumor bearing host was due to withdrawal by the tumor of some specific autogenous nutrients essential to life was first suggested by Moreschi[14] in 1909. Recently, Mider also raised the possibility that the host dies when tissues cannot supply further nitrogen to the increasing demand of a growing tumor which functions virtually as a "nitrogen trap."[15] However, Begg and Dickenson[16] Mider[17] and Stewart and Begg[18] found that even if rats bearing Walker carcinoma 256 were force fed to maintain their body weight, the characteristic alterations in liver catalase, adrenal weight and concentration of blood hemoglobin associated with malignant growth in rats were not prevented. Similarly in human subjects, Homburger and Young[19] were unable to alter significantly the hypoalbuminemia in any of their patients with gastric malignancies even when they were observed to retain enormous quantities of nitrogen during supplementary protein feedings.

The concept that a growing neoplasm traps essential foodstuff with resulting host starvation is inadequate to explain the profound metabolic alterations which our studies demonstrate in patients with cancer. The nitrogen balance was observed to become more positive in our patients during forced feeding but as the supplementation was maintained there was a definite tendency for the positive balance to approach equilibrium again, and a negative balance developed uniformly following the period of forced feeding. This suggests that patients with cancer handle increases in dietary nitrogen as if they had no previous pronounced deficit. The marked wasting of muscle mass in terminal cancer is apparent on even the most gross inspection of such patients and certainly does not correlate with our experimental findings, for if the malnutrition seen in cancer were a simple depletion process, these patients should have retained nitrogen as strongly as do patients with "simple" starvation.[20,21] Metabolic balance data during induced regression or destruction of tumor in man has been reported to show that fairly large quantities of nitrogen released by the tumor may be reutilized by the host tissues.[4] If this be true an interesting possibility suggested by these data and the results of our experiment is that the repletion of host tissues, even with an abundant supply of nitrogen and calories, could not occur because of some restraining influence exerted by the growing tumor on the host. Therefore, only when such an influence is removed, albeit temporarily, by some direct attack on the tumor itself can one expect a significant and sustained nitrogen repletion to occur.

The body weight data tend to support such an hypothesis. That we could produce a substantial increase in body weight by forced feeding appeared to us at first glance to be very encouraging. The disturbing facts, however, were the rapidity with which this increment in body weight was lost as soon as forced feeding was discontinued, and the large contribution to this increment made by an increase in intracellular fluid as determined by indirect calculations. This again suggests that some factor was operating during the time we were attempting to replete host tissues which would not allow the normal deposition, or at least a lesser rate of utilization, of body fat stores when the caloric intake was increased.

The observed simultaneous retention of nitrogen, potassium and phosphorus (the demand for the latter apparently at times great enough to lead to withdrawal of additional quantities of phosphorus from bone) indicates that some increase in protein anabolism occurred during the period of forced feeding. How much of this new protein was used for repletion of host

tissues and how much was incorporated into the tumor cannot be ascertained from our data but certainly is of utmost importance. Obviously, little is to be gained if all the extra nitrogen retained by a cancerous patient is utilized to produce more tumor tissue. Certain of our data suggest that there was an acceleration of the malignant process coincident with forced feeding in some of these patients. In this connection it is interesting that the one patient (A. M.) who was in significant negative nitrogen balance before forced feeding was the only one who continued to store nitrogen avidly throughout the period of forced feeding. (Fig. 1.) This suggests that an element of starvation, aside from that peculiar to malignant disease, was present in this patient.

That the presence of a progressively growing neoplasm in some way increases the caloric expenditure of the host[1,2] has also been suggested by the finding of elevated basal metabolic rates in many cancer patients with a wide variety of neoplasms (excluding the well known increase associated with leukemia and lymphoma)[22,23] and by the Newburgh technic for estimating caloric expenditure.[3] The latter method is admittedly a very indirect one. However, it certainly appears that comparative caloric expenditure values are valid whereas the absolute values, in some patients may be of less significance. Some direct determinations of caloric output on cancer patients are needed to settle this point.

Our present data on caloric expenditures and basal metabolic rates again support the impression that the caloric expenditure in patients with cancer is increased. The pertinent question, then, immediately becomes: Why and how does this come about? No definite answer to this question is possible at this time, but it does invite certain interesting speculations.

In most cancer patients in whom this elevation in basal metabolic rate and caloric expenditure has been observed, the quantity of malignant tissue present certainly does not appear to account for the magnitude of values obtained.[24] The host tissues themselves must contribute the bulk of the energy expenditure, secondary to some peculiar stimulus invoked by the growing tumor. Although it is stated that the basal metabolic rate in pregnancy rises during the latter months of gestation,[25] Newburgh found that the caloric expenditure, as determined both by the insensible weight loss method and by direct chamber measurement, was normal in a young woman in the last trimester of pregnancy.[5] Evidently, the presence of any rapidly growing tissue *per se* will not produce this particular response.

In a recent review of the subject of energy and nitrogen metabolism in cancer, several suggestions were offered as to how such an increase in caloric expenditure might occur.[9] Alterations in pathways of intermediary metabolism secondary to demands of the neoplasm (especially in the face of an inadequate supply of exogenous building blocks) and the possibility that the host is deprived of a diurnal reduction in energy expenditure, incident to continuous growth of the neoplasm throughout the day, were among the suggestions offered. It is conceivable that tumor tissue, being virtually a "nitrogen trap," demands only certain "building blocks" and forces the host's normal tissues to prepare them from either an exogenous or endogenous source at the expense of a greatly increased caloric expenditure, since it must also carry on its own vital functions at the same time.

Greenstein[26] has reviewed the evidence for the presence of a substance liberated by tumor tissue which specifically affects liver catalase in the host. If growing tumors do, in fact, liberate biologically active substances, it is also possible that this or some other factor elaborated by a growing neoplasm could have "dinitrophenol-like" effects on the host with its associated increase in caloric expenditure. In any event, the failure of dietary measures alone to replete our patients is understandable since such measures do not alter the basic malignant process. This again points out clearly that in cancer we are dealing with a systemic disease and not a local one.

As judged from the caloric expenditure measurements, basal metabolic rates and clinical response of the patients studied by us, it appeared that patients with progressively growing malignancies fall into two general categories, when one of the factors responsible for their characteristic cachexia, i.e. anorexia, was corrected by forced feeding. In one group such supplementation evidently did not significantly nor persistently disturb the established host-tumor relationship. (Fig. 1.) Clinical improvement, if any, was minimal and transient, and lasted only as long as the high intakes were maintained. Perhaps temporary repletion of body tissues occurred but there appeared to

be a rapid return to the pre-existing clinical state.

In the other group of patients (Figs. 2 and 5) forced feeding seemed capable of disturbing the balance which had been established between host and tumor, to the apparent detriment of the host. In the patient who could be followed closely for a long period of time the change, once produced, was apparently irreversible. Clinical evidence suggested acceleration of the malignant process during and after forced feeding in this group of patients, and the basal metabolic rate and caloric expenditure determinations also indicated this. The factors that contribute to this type of response are not known. Conceivably it could be the type of tumor involved, the stage in the natural history of the malignant process, or the nutritional state of the host at the time of forced feeding.

SUMMARY

1. Nine patients with growing malignancies were studied on a metabolism ward while nutritional rehabilitation was attempted by forced feeding. Balances of nitrogen, phosphorus, calcium, potassium, sodium and chloride were conducted before, during and after such treatment and repeated determinations of basal metabolic rate and calculations of caloric expenditures were made.

2. Characteristically, large gains in body weight were produced during forced feeding but weight loss was rapid when this was discontinued. An analysis of the weight gain in terms of body compartments revealed that the increase was predominantly due to an accumulation of large quantities of intracellular fluid.

3. Although there was initial retention of a significant quantity of nitrogen and phosphorus during forced feeding, there was a tendency for the nitrogen balance to approach equilibrium rapidly, and during the subsequent control periods a negative balance was uniformly observed. This suggests that in these patients a host repletion process could not be sustained.

4. Transient clinical benefit occurred in a few of the patients studied, while caloric expenditure determinations, basal metabolic and clinical data indicated that in about half the patients forced feeding had a detrimental effect.

5. The significance of the observed changes in nitrogen balance, the basis for the increased caloric expenditure in cancer, and the possible factors responsible for the different responses observed during forced feeding are discussed.

Acknowledgment: This work was done by the senior author while a Fellow of the American Cancer Society. He wishes to acknowledge the help of Dr. E. H. Keutmann during that time.

REFERENCES

1. Mider, G. B., Fenninger, L. D., Haven, F. L. and Morton, J. J. The energy expenditure of rats bearing Walker carcinoma 256. *Cancer Research*, 11: 731, 1951.
2. Mider, G. B., Sherman, C. D., Jr. and Morton, J. J. The effect of Walker carcinoma 256 on the total lipid content of rats. *Cancer Research*, 9: 222, 1949.
3. Waterhouse, C., Fenninger, L. D. and Keutmann, E. H. Nitrogen exchange and caloric expenditure in patients with malignant neoplasms. *Cancer*, 4: 500, 1951.
4. Fenninger, L. D., Waterhouse, C. and Keutmann, E. H. The interrelationship of nitrogen and phosphorus in patients with certain neoplastic diseases. *Cancer*, 6: 930, 1953.
5. Newburgh, L. H., Johnson, M. W., Lashmet, F. H. and Sheldon, J. M. Further experiences with the measurement of heat production from insensible weight loss. *J. Nutrition*, 13: 203, 1937.
6. Lavietes, P. H. The metabolic measurement of water exchange. *J. Clin. Investigation*, 14: 57, 1935.
7. Newburgh, L. H. In Williams, R. H.: Textbook of Endocrinology, p. 704. Philadelphia, 1950. William B. Saunders.
8. Lavietes, P. H., D'Esopo, L. M. and Harrison, H. E. The water and base balance of the body. *J. Clin. Investigation*, 14: 251, 1935.
9. Fenninger, L. D. and Mider, G. B. Energy and nitrogen metabolism in cancer. *Advances Cancer Research*, 11: 229, 1954.
10. Keys, A. and Brozek, J. Body fat in adult man. *Physiol. Rev.*, 33: 245, 1953.
11. Reifenstein, E. C., Jr., Albright, F. and Wells, S. L. The accumulation, interpretation and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus and nitrogen. *J. Clin. Endocrinol.*, 5: 367, 1945.
12. Boyd, E. M., Boyd, C. E., Hill, J. G. and Ravinsky, E. The lipid and water content of carcass, skeletal muscle, and testicle in the host component of the albino rat Walker carcinoma 256 dual organism at progressive stages of tumor growth. *Canad. J. Biochem. and Physiol.*, 32: 359, 1954.
13. Stewart, A. G. and Begg, R. W. Systemic effects of tumors in force-fed rats. III. Effect on the composition of the carcass and liver and on the plasma lipids. *Cancer Research*, 13: 560, 1953.
14. Moreschi, C. Beziehungen zwischen Ernährung und Tumorwachstum. *Ztschr. f. Immunitätsforsch u. exper. Therap.*, 2: 651, 1909. Cited by Mider et al.[15]
15. Mider, G. B., Tesluk, H. and Morton, J. J. Effects of Walker carcinoma 256 on food intake, body weight and nitrogen metabolism of growing

rats. *Acta de l'union inter. contre le cancer*, 6: 409, 1948.

16. Begg, R. W. and Dickenson, T. E. Systemic effects of tumors in force-fed rats. *Cancer Research*, 11: 409, 1951.
17. Mider, G. B. Some aspects of nitrogen and energy metabolism in cancerous subjects: a review. *Cancer Research*, 11: 821, 1951.
18. Stewart, A. G. and Begg, R. W. Systemic effects of tumors in force-fed rats. II. Effect on the weight of carcass, adrenals, thymus, liver and spleen. *Cancer Research*, 13: 556, 1953.
19. Homburger, F. and Young, N. H. Studies on hypoproteinemia. I. Hypoproteinemia in patients with gastric cancer; its persistence after operation in the presence of body tissue repletion. *Blood*, 3: 1460, 1948.
20. Keys, A., Brozek, J., Henschel, A., Mickelson, O. and Taylor, H. L. The Biology of Human Starvation, p. 387. Minneapolis, 1950. Univ. of Minnesota Press.
21. Strang, J. M., McCluggage, H. B. and Brownlee, M. A. Metabolism in undernutrition; its changes during treatment by high caloric diet. *Arch. Int. Med.*, 55: 958, 1935.
22. Silver, S., Poroto, P. and Crohn, E. B. Hypermetabolic states without hyperthyroidism (nonthyrogenous hypermetabolism). *Arch. Int. Med.*, 85: 479, 1950.
23. Terepka, A. R., Fenninger, L. D. and Waterhouse, C. Unpublished observations.
24. Gellhorn, A. and Holland, J. F. Neoplastic disease: medical care in advanced cancer. *Ann. Rev. Med.*, 5: 183, 1954.
25. McCann, W. S. Calorimetry in Medicine, p. 18. Baltimore, Williams & Wilkins Co., 1924.
26. Greenstein, J. P. Biochemistry of Cancer, p. 529, 2nd ed. New York, 1954. Academic Press.

Review

The Porphyrins and Porphyria*

A Review of Eighty-one Cases

WILLIAM J. MARTIN, M.D., *and* FRANK J. HECK, M.D.
Rochester, Minnesota

THE porphyrins are pigments composed of four pyrrole rings connected by four methene bridges. Porphyrins differ depending on the character of the substituent groups on the eight free corners of the four pyrrole nuclei. The porphyrins are fundamentally related to cellular metabolism. Hemoglobin, myohemoglobin, cytochrome and catalase are porphyrin-protein compounds. Chlorophyll is a magnesium-porphyrin compound.

Porphyria is a rare metabolic fault in which abnormal amounts and kinds of porphyrins are excreted in the urine and feces. These include coproporphyrin I, coproporphyrin III, uroporphyrin I, Waldenström's uroporphyrin and porphobilinogen. The coproporphyrins are excreted in detectable amounts by normal subjects but the other compounds are not demonstrable by ordinary laboratory methods in the secretions of normal persons. The presence of uroporphyrin in normal urine can be demonstrated by more precise methods.[1] Other porphyrins, such as protoporphyrin and deuteroporphyrin which are present in increased amounts in the stools of patients with gastrointestinal bleeding,[2] ordinarily have no importance in establishing the diagnosis of porphyria. However some investigators[3,4] have suggested that large quantities of protoporphyrin and coproporphyrin in the stool during periods of remission are characteristic of the mixed or chronic form of the disorder. Broadly speaking, the diagnosis of porphyria is established by the demonstration by qualitative methods of one of the uroporphyrins or of the colorless chromogen porphobilinogen in the urine.

Hoppe-Seyler[5–7] first clearly described a porphyrin and its preparation *in vitro* from hemoglobin (hematoporphyrin). The term "hematoporphyrinuria," which is still used in some quarters, is obsolete in that hematoporphyrin[5,6] does not occur in nature, but is a "laboratory" porphyrin. Hans Fischer and co-workers[8–10] in 1913 demonstrated the distinction between hematoporphyrin and the naturally occurring porphyrins. Fischer isolated from the excreta of a patient with congenital porphyria both crystalline coproporphyrin and crystalline uroporphyrin. He isolated coproporphyrin from both the feces and the urine and uroporphyrin from the urine. Despite his belief to the contrary, the feces of patients with porphyria also contain uroporphyrins,[11] although usually in smaller amounts than are found in the urine. Later Fischer crystallized and synthesized protoporphyrin,[12] which is the porphyrin of the hemoglobin molecule.

In 1928 van den Bergh and Hyman[13] demonstrated that erythrocytes contain free protoporphyrin. The concentration of protoporphyrin in the red blood cells is elevated in the presence of iron deficiency, of anemia and of conditions such as lead poisoning which may interfere with the utilization of iron in synthesis of hemoglobin.[1] Very small amounts of coproporphyrin also are present in erythrocytes;[14] as is true of protoporphyrin, coproporphyrin also is especially concentrated in the reticulocytes. Conditions associated with increased synthesis of hemoglobin are characterized by increased concentration of coproporphyrin in the erythrocytes. Coproporphyrins of type I and type III are excreted normally in the feces and urine, although 60 to 80 per cent of the coproporphyrin normally is of type I.[15] Coproporphyrin of type III is considered by some investigators to

* From the Section of Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

be a normal precursor of the protoporphyrin of hemoglobin, but coproporphyrin of type I is thought to be merely a side product of the synthesis of hemoglobin.[16] The white matter of the nervous system of warm-blooded animals contains small amounts of coproporphyrin of type III apparently formed there *in situ;* the absence of this porphyrin from the nervous system of cold-blooded animals may indicate its relationship to regulation of temperature.[16]

Uroporphyrin I[10] is found in association with congenital porphyria. Waldenström's uroporphyrin,[17–19] which is mainly composed of an isomer of uroporphyrin type I, is found in cases of intermittent acute porphyria and in cases of mixed (chronic) porphyria. No demonstration has been made in nature of uroporphyrin of type III.[16] Recently uroporphyrin has been found to increase in the urine following exposure to lead.[1]

Porphobilinogen is a colorless, non-fluorescent monopyrrole which occurs in persons with porphyria and is characterized by abdominal pain or neurologic disturbances.[16] It is not found in association with the congenital type of porphyria.

THE THREE TYPES OF PORPHYRIA

1. Congenital Porphyria (*Porphyria erythropoietica*). Congenital porphyria was first described by Günther in 1911.[5,6] It is inherited as a recessive mendelian characteristic.[20] Males are affected twice as frequently as females. It may be noted at birth, in infancy or early childhood. It appears to reflect a constitutional fault in the synthesis of hemoglobin from porphyrin.[21] The amount of porphyrin in developing normoblasts in patients with this disease is excessive.[21]

The color of the urine varies from pink to red to black, depending on the concentration of porphyrins and the state of oxidation. The urine may deepen in color on standing if it is kept acid; oxidation and development of color are prevented by alkalinity.[20]

Pink to brown discoloration of the enamel of the teeth may occur (erythrodontia). In some cases erythrodontia is not evident but under ultraviolet light the teeth may exhibit a definite red fluorescence due to the presence of the porphyrins. Such fluorescence may also be detected in the fingernails and in samples of urine of these patients.

Occasionally the bones of the hand are pigmented sufficiently to be demonstrated by transillumination.[20] Deposition of porphyrin in developing bones and teeth probably is related to its physical affinity for calcium phosphate.[21] In this type of porphyria, skin lesions resulting from exposure to the sun vary from erythema to formation of vesicles and large bullae (hydroa aestivale, urticaria solaris).[20,22] In some instances the skin lesions are of a more eczematoid nature (eczema solare).[21] Subsequent infection of these lesions may eventuate in extensive scarring and mutilation. Digits as well as parts of the nose and ears may be lost entirely. Ectropion, together with scarring of the conjunctiva and cornea, may result.[21] The skin lesions are seen most commonly over the face, neck and hands. Hyperpigmentation and hypertrichosis may be noted, especially on exposed areas.[22,23] Experiments indicate that ultraviolet light enhances formation of porphyrin;[2] clinical observations suggest that formation and excretion of porphyrin may increase in relation to increased exposure to light.[2]

Hepatosplenomegaly may be detected on examination of older children. Hemolytic anemia is not infrequent among them. Splenectomy may result in prompt and marked, although perhaps transient, reduction in formation of porphyrin.[16]

Porphyrins characteristically excreted in association with the congenital type of porphyria are uroporphyrin I and coproporphyrin I. Porphobilinogen is not demonstrable in this form of the disease.

2. Intermittent Acute Porphyria (*Acute Toxic or Idiopathic*). Intermittent acute porphyria is inherited as a mendelian dominant[20] and affects females twice as frequently as males.[21] It is most likely to become manifest when patients are in the age group of twenty to forty years inclusive. Unlike the congenital type, the acute variety manifests individual attacks with varying periods of remission.

The intermittent, acute type of the disease may be manifested by paroxysms of severe abdominal pain which are usually of the nature of colic and which may be localized or generalized. Spasm and rebound tenderness are not common[21] but nausea and vomiting are usual. The leukocyte count usually is normal but it may be considerably elevated. Not infrequently abdominal crises of porphyria lead the unwary surgeon to perform useless laparotomy.[22] Such abdominal pains often have led to erroneous diagnoses of

appendicitis, biliary or renal colic, intestinal obstruction, and so forth.

Acute porphyria may be manifested in any part of the nervous system—central, peripheral or autonomic. Neurologic findings may simulate those of a wide variety of conditions, including encephalitis, poliomyelitis, polyneuritis and poisoning from heavy metals. Sensory changes generally are absent, although slight hypesthesia and paresthesia may be present. Signs of upper motor neuron changes are usually absent. Coma and convulsions may occur. Optic atrophy, palsy of ocular muscles and dysphagia may result from involvement of cranial nerves. Hoarseness due to weakness of vocal cords may be noted. Various forms of paralysis, including flaccid quadriplegia, have been noted. Wrist or foot drop is not an uncommon occurrence. Atrophy and contracture of muscles may develop. Pain (especially in the legs), similar to that of diabetic neuropathy, may precede paralysis.[21] The possibility exists that the abdominal pains are attributable to involvement of the autonomic nervous system rather than to the direct effect of porphyrins on the intestinal wall.[2]

Acute attacks may be preceded for many years by undue nervousness, neurasthenia or mild hysteria. During the attack, marked hysterical behavior, manic depressive or Korsakoff's psychosis and delirium may occur.[21]

Hypertension is a frequent occurrence among these patients and they are frequently plagued by severe constipation. Both hypertension and constipation may disappear completely during remissions. During the periods of hypertension spastic changes in the retinal arteries may be noted,[2] and there may be associated temporary loss of vision.[1]

Tachycardia during an acute episode of the disease is common. Photosensitivity is not a manifestation of acute porphyria.

It is well to remember that acute attacks may prove fatal. One mechanism of death is ascending paralysis with respiratory failure.

The urine may be of normal color but may darken when left standing. The characteristic porphyrins excreted in the acute type are Waldenström's uroporphyrin and coproporphyrin III; the monopyrrole, porphobilinogen, is also excreted. In certain patients the attack and associated porphobilinogenuria may be very brief, and during remission examination of the urine may fail to reveal porphyrin. Thus the urine should be examined at the height of the attack if at all possible. The test for porphobilinogenuria constitutes the most useful screening test for this disease.

3. Mixed or Chronic Porphyria (Cutanea tardive). Just as in cases of the acute type, abdominal pains and neurologic disturbances may occur in cases of mixed, or chronic, porphyria. However, unlike the patient with the acute type of the disease, the patient with chronic porphyria may exhibit photosensitivity; but, as in the acute type, onset of the disease is rare before puberty. The usual age of onset is from forty to sixty years of age, inclusive. The cutaneous lesions are considerably less mutilating in this condition than they are in congenital porphyria.

Skin vesicles may also appear after trauma and on exposure to heat.[22,23] Since skin lesions are not noted until adult life, this type of porphyria has been called "cutanea tardive." Occasionally the exposed skin is toughened, as it is in scleroderma.[23] The patients are sometimes mistakenly considered to have chronic eczematoid dermatitis.

Other manifestations can be briefly mentioned: Pigmentation may occur in the form of diffuse melanosis of the exposed skin and hair. Discrete pigmented macules also may occur.[23] Brunsting and his co-workers[22,23] have emphasized the so-called "purplish facies." A peculiar violaceous hue or suffusion may occur, such as that noted in alcoholics or victims of polycythemia. The conjunctivae may also be suffused, giving the patient a bleary-eyed and dissipated look. Frequently liver function is impaired.[23] Jaundice is not infrequent. Frank cirrhosis may result. Hypertrichosis is especially striking in women.[23] Diabetes mellitus is not an uncommon associated finding.[2] Characteristic porphyrins excreted in the mixed type of porphyria are the same as those excreted in the acute type. However, porphobilinogen is not so commonly observed as it is the acute type.

Because the liver is principally responsible for the abnormal formation of porphyrin in the acute and in the chronic (mixed) types, some authors group types 2 and 3 under the term "porphyria hepatica."[2]

CONDITIONS EXCLUDED FROM THE THREE TYPES

"Secondary coproporphyrinuria" should be sharply distinguished from the various types of porphyria. The term, "secondary coproporphyrinuria," refers to those states during which

the patient excretes increased amounts of coproporphyrins in the urine. The uroporphyrins and porphobilinogen are not excreted, and the clinical picture of porphyria is absent. In Table I are listed the states associated with secondary coproporphyrinuria, classified according to the type of coproporphyrin excreted. "Idiopathic coproporphyrinuria" is found on examination of a small number of otherwise normal persons in whom excretion of urinary coproporphyrin is markedly increased.

TABLE I
TYPE OF COPROPORPHYRIN EXCRETED IN VARIOUS STATES[16–20] ASSOCIATED WITH SECONDARY COPROPORPHYRINURIA

Coproporphyrin I	Coproporphyrin III
Acute febrile states (pneumonia, lung abscess, rheumatic fever, etc.)	Poliomyelitis
Infectious hepatitis	Hodgkin's disease
Obstructive jaundice	Portal cirrhosis
Pernicious anemia, hemolytic anemia and leukemia	Aplastic anemia
	Metal and chemical poisoning

A certain amount of coproporphyrin is excreted normally. Higher values for urinary coproporphyrin usually are found among males than among females; normal males excrete 100 to 300 μg. per day; normal females, 75 to 275 μg. per day.[1] Approximately half of the freshly passed urinary coproporphyrin is excreted as a colorless non-fluorescent precursor compound which is convertible to fluorescent coproporphyrin by iodine, air, peroxide or ultraviolet light. Values for urinary coproporphyrin are depressed in the presence of renal damage.[1] Excess amounts are encountered on examination of the following persons who do not have porphyria: otherwise normal persons who recently have imbibed more than a moderate amount of alcohol, victims of "idiopathic coproporphyrinuria" and persons with underlying disease which induces secondary coproporphyrinuria. Some evidence exists that determination of urinary coproporphyrin can be used as a screening test for exposure to lead. The presence of increased values for porphyrin found upon examination of workers exposed to lead, in the absence of high excretion or of high blood concentration of lead, has been suggested as an early sign of lead absorption. Similar phenomena may apply with relation to other heavy metals and to certain chemicals.

REVIEW OF CASES

Eighty-one cases of porphyria were seen at the Mayo Clinic from 1935 to 1953 inclusive. Of this number only one was of the so-called congenital variety. Of the remaining eighty cases of intermittent acute or of chronic (mixed) porphyria, which together are known as "porphyria hepatica," thirty-eight were classified as of the acute intermittent type, and forty-two were thought to represent the chronic (mixed) type. In the general literature on the subject the acute type is said to occur more frequently than the chronic type.[2] However, Brunsting and his co-workers[22,23] have described some of the latter patients from the point of view of their dermatologic manifestations, and the relative frequency of the two types in our series may reflect the high index of suspicion of porphyria on the part of local dermatologists. Also to be considered is the fact that possible cases are referred to these investigators, owing to their known interest in the condition. There appeared to be a striking age difference between patients who had the acute type and those who had the chronic type: three-fourths of the patients who had the acute type were less than forty years old, and three-fourths of those who had the chronic type were more than forty years old.

Forty-two of the eighty patients excreted porphobilinogen, a uroporphyrin and excessive coproporphyrin. (Table II.)

In twenty-nine cases porphobilinogen was not detected; five of these cases were studied before it became customary to test for porphobilinogen. Excretion of porphobilinogen was not completely correlated with involvement of the central nervous system and abdominal complaints. In nine patients who exhibited severe involvement of the central nervous system porphobilinogen was not excreted. Six patients who had abdominal crises also did not excrete porphobilinogen. In one patient both involvement of the central nervous system and abdominal crises occurred; in this patient a failure to excrete porphobilinogen was noted. Six patients (Table II), in whom porphobilinogen was pro-

duced and coproporphyrinuria revealed, failed to excrete a uroporphyrin. Waldenström[17–19] also observed cases of porphyria in which porphobilinogenuria was definitely present but in which no uroporphyrin was noted.

In forty-three cases no agent was suspected of inducing a porphyric crisis. The agents thought to have precipitated symptoms in susceptible patients are listed in Table III. The fact that the number of patients given in Table III adds up to forty-three is not a mistake; in several cases more than one agent was suspected. For instance, in three cases both barbiturates and alcohol apparently were factors in the condition. In one case both arsenic and alcohol were thought to be responsible for exacerbation of the porphyria.

TABLE II
URINARY EXCRETION OF PIGMENTS IN EIGHTY CASES OF PORPHYRIA HEPATICA (CASES DISTRIBUTED TWO WAYS)

Pigments	Cases
Uroporphyrin (alone or otherwise)	71
Porphobilinogen (alone or otherwise)	51
Porphobilinogen, uroporphyrin and coproporphyrin	42
Uroporphyrin and coproporphyrin without porphobilinogen	28
Porphobilinogen and coproporphyrin without uroporphyrin	6
Only porphobilinogen	3
Only uroporphyrin	1

TABLE III
AGENTS THOUGHT TO HAVE PRECIPITATED SYMPTOMS

Agent	Patients*
Alcohol	23
Barbiturates	12
Sulfonamides	2
Surgical procedures	4
Arsenic	2

* In several cases more than one agent was suspected; therefore this column adds to more than the thirty-seven patients whose symptoms were thought to be attributable to the agents.

Pathologic changes have been demonstrated in the livers of victims of porphyria. In thirty-two patients of our series the bromsulphalein test of liver function revealed retention of the dye, grade 2 (12 to 22 per cent) or more, in one hour; the quantity of bromsulphalein administered was 5 mg. per kg. of body weight. In thirty-three cases results of the bromsulphalein test were within normal limits. In one additional case a value for serum bilirubin of 19.8 mg. per 100 cc. prevented performance of a dye test. The question has been raised whether the porphyrins, which are pigments, might provide a basis for a falsely positive bromsulphalein reaction; however, results of the dye tests cannot be exactly correlated with pigmentation of the skin and discoloration of the urine. To be responsible for a falsely positive bromsulphalein reaction enough porphyrin would have to be present to impart a definite red color to the plasma. If plasma so colored occurs, it must be very rare.

TABLE IV
NOTABLE MANIFESTATIONS IN EIGHTY CASES OF PORPHYRIA HEPATICA

Manifestation	Cases*
Abdominal "crises"	44
Discolored urine	41
Emotional disturbances	36
Involvement of the nervous system†	31
Pigmentation	29
Erythremia	12
Hypertension	12
Hirsutism	5
Severe constipation	5

* In many cases manifestations numbered more than one; therefore, the total of this column is more than eighty.

† See Table v for breakdown.

In thirty-one patients the nervous system was involved. (Tables IV and V.) Some amplification might be helpful. Of the eight patients who had convulsive disorders, one had jacksonian seizures of the left leg and one had facial palsy. Peripheral neuritis in six patients was accom-

panied by paresthesia and pain in the limbs. Two patients bitterly complained of insomnia.

Of the forty-four patients in whom abdominal "crises" occurred the pain usually was paroxysmal and was generalized more often than localized. When localized, it caused confusion with the disease states ordinarily associated with the portion of the abdomen concerned. When generalized and colicky, and associated with constipation, it was confused with the pain of intestinal obstruction; in instances wherein roentgenograms of the abdomen demonstrated distention of the colon with trapped gas, this confusion was compounded. Spasm and rebound tenderness usually were not associated with abdominal pain. Vomiting was correlated with the severity of the pain. In less than 10 per cent of instances of such "crises" the leukocyte count was elevated. Severe episodes of pain were much more often noted in patients considered to have acute than among those presumed to have chronic porphyria. Patients who had the chronic type of porphyria were more likely to have less distressing abdominal pain than those who had the acute type; occasionally a patient with chronic porphyria had a sudden and severe "crisis."

TABLE V

MANIFESTATIONS OF INVOLVEMENT OF NERVOUS SYSTEM IN THIRTY-ONE CASES IN WHICH SUCH INVOLVEMENT WAS NOTED

Type of Involvement	Cases*
Diffuse (encephalomyelitis, polyneuritis, ascending paralysis)†	14
Convulsive disorder	8
Peripheral neuritis	6
Episodic stupor	5
Hoarseness	5
Hemianopsia	1
Periodic blindness	1
Facial palsy	1

* In several cases types of involvement numbered more than one; therefore, the total of this column is more than thirty-one.

† Two of these patients died from bulbar palsy.

Pigmentation was evident in twenty-nine patients; this diffuse melanosis of exposed areas was the chief reason why most of them came to the clinic. Frequently the hair which grew in areas of pigmentation was pigmented too.

Other features which either are not listed in Table IV or which require comment are (1) hirsutism, (2) concentration of hemoglobin, (3) emotional disturbances, (4) hypertension, (5) associated disease and (6) familial porphyria. The five patients who were hirsute were markedly so on exposed areas; all were women. The concentration of hemoglobin in the blood of twelve patients exceeded 15 gm. per 100 cc. All of these patients had the "violaceous facies." The value for hemoglobin of four patients was less than 10 gm. per 100 cc. of blood. Emotional disturbances of thirty-six patients were worthy of note. Three patients were institutionalized for this reason; one had been given shock treatment. Twelve patients were consistently hypertensive; that is, their diastolic pressures were in excess of 100 mm. of mercury. In three patients syphilis and in four, diabetes mellitus were associated diseases. In eight cases other members of the patient's family were also known to have excreted abnormal porphyrins.

Eight patients died. Bulbar paralysis, cirrhosis of the liver, hepatic abscesses with septicemia attributable to Escherichia coli, and carcinoma of the stomach with metastasis were the four apparent causes of death in the five cases in which necropsy was performed.

ILLUSTRATIVE CASES

CASE I. A white woman, twenty-five years of age, came to the clinic in July, 1945, with a chief complaint of "spells" of abdominal pain of two years' duration. She also complained of pain in the extremities. She stated that about one year previously she had had her first spell of severe, generalized abdominal pain, associated with burning on urination and pronounced general weakness. She described rather marked urethral tenesmus during these spells. One month after the initial spell she had had the same kind of seizure again and then had been free of such seizures for the next six months.

In December 1944, these symptoms had recurred, together with "shooting pains" and soreness in the muscles of all four extremities. She then had been free of symptoms until June 1945, when she had had another episode, associated with the passage of urine which initially had been red but which had turned black when left standing. She also recalled that in January 1945, after she had undergone appendectomy, her voice had failed and she had suffered con-

siderably from general weakness. When we saw her she still was unable to speak above a whisper and even then her voice became fatigued rapidly. She had been constipated rather markedly since the onset of her complaints.

On physical examination, note was made of marked weakness of the extremities, hoarseness, generalized tenderness of the abdomen and soreness of the limb muscles on forced motion. Her condition improved after institution of a diet high in carbohydrates and proteins with vitamin supplements. However, at the time of her dismissal from the hospital there was still considerable weakness of the hands and wrists, most marked on the left. Slight weakness of the legs and feet was also present. During hospitalization she had complained of paresthesia of the feet and about the abdomen.

The patient's urine consistently contained porphobilinogen but the first two specimens examined did not contain uroporphyrin. Uroporphyrin was present in appreciable amounts later, however, even though the patient was clinically improved. Retention of bromsulphalein was graded 3 at the end of one hour.

The patient died at home shortly after dismissal from the clinic.

Comment. The dysuria, "red urine" and nonspecific abdominal pains suggested that primary disease of the urinary tract be considered in this case. A physician whom the patient had consulted previously, however, had determined that the urine did not contain blood and had made a presumptive diagnosis of porphyria. The paresthesia, muscular paresis, hoarseness, marked constipation and the intermittent course of the disease are worthy of emphasis.

Case II. A Negro man, twenty-two years of age, first was admitted to the Mayo Clinic in September 1945, with a complaint of abdominal pain. He stated that he had been well until a year and a half before when mid-abdominal distress had come on about 1:00 o'clock each afternoon. The pain would be somewhat relieved by drinking a glass of milk, despite the fact that he had eaten about an hour before. Gastric fluoroscopy had been performed elsewhere and had given ostensibly negative results. Nevertheless, the patient had been advised to follow a regimen for peptic ulcer. This regimen was thought to have provided partial relief for about four months but the pains had then returned and had been associated with belching, despite the patient's adherence to the measures to combat ulcer. Concurrent with return of the pain the patient had noted rather marked constipation. He also had begun to complain of insomnia. Roentgenograms had revealed a "sluggish, enlarged bowel." He had lost 50 pounds (about 23 kg.) in the eighteen months previous to our seeing him.

The night after his admission to the hospital he had severe pain above the pubis and in the flanks but the following morning the pain returned to the middle of the abdomen. He consistently excreted excessive quantities of coproporphyrin, Waldenström's uroporphyrin and porphobilinogen in the urine but the pigments were not found in the urine of his mother or in that of his sister Retention of bromsulphalein in the serum was graded 1 at the end of one hour. The patient's condition gradually improved and he left the hospital, at least temporarily free of his abdominal distress.

Comment. This man gave a history of abdominal pain which ostensibly was relieved by taking food. The marked loss of weight in this patient is unusual in our experience. The insomnia and constipation warrant emphasis.

Case III. A white man, thirty-one years of age, visited the clinic for the first time in January 1951, because of pain in the abdomen. A draft board had rejected him for service in the armed forces because it was thought that he had diabetes.

The patient related that he had had episodes of sharp, cramping pain in the lower part of the abdomen for the last eighteen years. These spells had lasted about two hours each time and usually had been accompanied by nausea and occasionally by vomiting. The urine during these episodes had been dark brown. However, he had never noticed that his stools had been light in color nor had he been aware of the presence of jaundice. Appendectomy had been performed in the course of the first attack because of the pain. Three years before we saw the patient this pain had begun to extend around to the back on the right side. Special roentgenographic procedures all had given negative results and laparotomy had been performed, without disclosure of anything noteworthy. During the postoperative period both upper extremities gradually had become paralyzed but the paralysis had improved until, after four or five months, paresis limited to the extensors of both hands and of all fingers had remained. The abdominal pain which brought the patient under our care had been present for three months, and he had resorted to taking considerable amounts of meperidine hydrochloride (demerol® hydrochloride) in attempts to gain relief. Also, in the three years before we had seen him he had been afflicted with a convulsive disorder which had recurred almost daily and had continued as attacks of two to five minutes each time.

At the time of initial physical examination noteworthy observations were marked atrophy of the muscles of both arms, bilateral wrist drop and paralysis of the extensors of the fingers. An excess of coproporphyrin and Waldenström's uroporphyrin and porphobilinogen were detected in the urine. On the day after the patient's admission, in the course of examination, the patient suddenly and slowly straightened into opisthotonos and a marked rhythmic twitching of the right side of the face developed but failed to extend to other parts of his body. Conjugate deviation of the eyes to the right was noted. As the seizure subsided the patient's head turned to the left,

the eyes underwent left conjugate deviation and, for a short time, there was rapid lateral nystagmus. Immediately thereafter a neurologist found corneal anesthesia, lateral deviation of the right eye, the aforementioned muscular atrophy and paralysis. Over the next ten days he had twelve to fifteen seizures which did not differ markedly from the one described. The patient then lapsed into status epilepticus and died.

Comment. We do not know why the patient was once considered to have diabetes unless perhaps the pigmented urine confused the reading of the Benedict reaction. The value for blood sugar with the patient fasting was within normal limits. Initially a diagnosis of sickle cell anemia was entertained because of the paroxysms of abdominal pain associated with the passage of dark urine. The two unproductive laparotomies, the postoperative paralysis, the convulsions and the mode of death are noteworthy.

CASE IV. A Negro man, thirty-four years of age, was first seen at the Mayo Clinic on March 23, 1953, with a chief complaint of abdominal pain. He had been well until four years previously when he had begun to have distress in the epigastrium; this distress had been relieved by the taking of food. At this time he had been under considerable emotional stress and had chronically overindulged in coffee and tobacco. The epigastric distress had stopped four months after it had begun and had not recurred.

Ten days before he came to Rochester the patient had begun to have episodic, intense, periumbilical cramps. He had obtained some relief by taking salicylates and by flexing his legs on his trunk. Five days before he had come to Rochester he had undergone gastric fluoroscopy but without disclosure of anything significant. Since the fluoroscopy the cramping had been intensified, so much so that when he had arrived in Rochester it had been necessary for him to call a physician immediately and obtain an analgesic. Despite the use of a considerable number of laxatives by the patient, he had not had a bowel movement for three days.

The pertinent aspects of physical examination included marked distention of the abdomen with occasional, high-pitched, tinkling sounds on auscultation. Throughout the examination the patient belched constantly. No rigidity and no tenderness at particular points was noted. About thirty-six hours after examination the patient experienced a sudden chill, which lasted twenty to thirty minutes. This was followed by profuse sweating. The temperature rose to 101°F. Considerable guarding of the abdomen was noted. Next day apparently fresh blood was obtained on gastric suction but the man's general condition remained about the same.

Four days after the patient's admission considerable tenderness was noted in the right upper quadrant of the abdomen; however, this abated in the next twenty-four hours. One week after his admission the patient's condition improved so much that he appeared almost euphoric. The urine, which had been dark and reddish brown, changed to a light, clear yellow. However, he complained of insomnia and apprehensiveness. At about this time he also began to complain of coldness, painful cramps and decreased ability to detect painful stimuli in the right lower extremity. The right knee reflex had been normal on initial examination but now became considerably depressed.

Episodes of dyspnea now appeared and the abdominal cramps recurred. Coincidentally he complained of thoracic distress and pain which prevented full expansion when he breathed. Shortly thereafter stiffness of the neck developed. Neurologic examination did not demonstrate any objective evidence of disease of the central nervous system. Restlessness and sleeplessness became progressively more marked but could be fairly well controlled with chloral hydrate and meperidine hydrochloride (demerol hydrochloride).

The urine was examined on nine occasions for evidence of porphyria. On the first eight examinations porphobilinogen was demonstrated in significant amounts but evidence of uroporphyrin was not found. On the seventh examination neither porphobilinogen nor uroporphyrin was detected. The eighth examination gave only a slightly positive reaction for porphobilinogen. The ninth examination gave a positive reaction for both uroporphyrin and porphobilinogen.

Other pertinent laboratory data included the finding of polychromatophilic stippling of red blood cells and myeloid immaturity (early myelocytes). Retention of bromsulphalein after one hour was 20 per cent. The finding of considerable intestinal gas in roentgenograms of the abdomen had caused confusion initially with regard to possible intestinal obstruction.

Five weeks after his admission to the hospital the patient died. The day before his death he had been somewhat delirious and had had several episodes of vomiting.

Comment. As in Case II this man was a Negro who gave a history of abdominal pain relieved by ingestion of food. The clinical course of the disorder in this patient raises the question as to whether or not porphyria is accompanied by actual intestinal obstruction or whether this disease of protean manifestations is capable of simulating intestinal obstruction so closely as to be indistinguishable. The passage of dark urine, the motor and sensory defects, the dyspnea and the mode of death appear worthy of emphasis. The significance of the gastrointestinal bleeding was not determined.

CASE V. A white, married woman, fifty-nine years of age, first visited the clinic in February, 1953. She had been a fur finisher for twenty-nine years. She had had fifteen siblings, most of whom had died in infancy of unrecognized cause. She had had three children by a prior marriage, all of whom had died relatively young of unrecognized cause. She had undergone

gynecologic surgery in 1927 and 1928. Her chief complaint was of a burning sensation of the skin which has been present for the last two and one-half years. When this had appeared she had noticed pigmentation of the exposed areas of her skin together with general malaise.

At home her physician had told her two and one-half years previously that she had anemia (hemoglobin 44 per cent). For this she had received injections of a liver preparation once or twice weekly and had taken a preparation of iron orally three times daily. Also about two and one-half years before she had begun to lose weight; finally the loss amounted to 30 pounds (13.6 kg.). She had noted that her general malaise always became more severe on exposure to sunshine. Further, such exposure initiated the eruption of multiple small vesicles and the eruption was associated with marked burning. One year before the patient's visit to the clinic she had noticed several times that her urine was very dark. At this time she had been hospitalized and a diagnosis of porphyria had been made. The patient's mother had diabetes.

In 1930 the patient had received a roentgenologic diagnosis of peptic ulcer which was difficult to evaluate because she also complained of having attacks every seven to ten days of rather intense, colicky abdominal pain. The burning sensation was most marked in the face, was not constant, but was very severe. The burning was less in the arms and over the scalp. Nevertheless, because of it the woman sometimes refrained from combing her hair. She volunteered the information that she became, as she said, "very sick to my stomach" whenever she used sleeping tablets.

The patient was thin and elderly, and exhibited marked bronze pigmentation, particularly on exposed surfaces and the thorax. In addition to diffuse "tanning," mottled areas of deeper pigmentation were noted. Scattered about the neck were minute areas of depigmentation. Hypertrichosis also was marked, especially on the face, including the forehead and the area which in the male is bearded; hypertrichosis of the extremities was less. Suffusion in the exposed areas was distinct. The skin on the sides of the neck and in the intermammary area was thickened and somewhat suggestive of scleroderma. The liver extended four fingerbreadths below the right costal margin.

The color of the urine varied from dark amber to brownish pink. On three occasions uroporphyrins were detected but no evidence was present that porphobilinogen was excreted. On one occasion, when a quantitative determination was made, 5.16 mg. of coproporphyrin per day were found in the urine.

The patient was dismissed to follow a diet high in carbohydrate and protein and she was advised to use vitamin supplements. She was cautioned to remain out of the sunlight and not to use barbiturates. She did not use alcohol. Later the pigmentation and hirsutism subsided and the paresthesia decreased markedly. The improvement ostensibly was attributable to the diet.

Comment. The early deaths from unknown causes of the patient's large number of siblings and of her three children raise the question as to whether or not porphyria is attributable to a familial, potentially lethal process. The patient's declaration that exposure to the sun made her malaise more severe and that barbiturates were capable of provoking vomiting was adequate basis for considering a diagnosis of porphyria, especially in view of a history of passage of dark urine. The degree of pigmentation and hypertrichosis was striking. Although the woman was concerned about the cosmetic defects invoked by her disease, the intensity of the paresthesia was her main reason for coming to the clinic. The improvement was sufficiently marked to encourage the examining physicians to recommend similar treatment to other victims of chronic (mixed) porphyria.

CASE VI. A white woman, sixty-one years of age, first visited the clinic in 1927 with complaints of enlargement of the abdomen and nervousness. A year and a half before that visit she had received a diagnosis of nervous exhaustion for a condition that was characterized by severe backache, extreme lethargy, loss of 15 pounds (6.8 kg.), fever, insomnia and nervousness. She had gradually recovered from the nervous exhaustion over a period of six months. Three months before her visit to Rochester she had begun to note a vague feeling of fullness in the lower part of the abdomen, nausea early in the morning and bloating. On physical examination at the clinic in 1927 the only abnormal condition found had been enlargement of the uterus. Uterine myomectomy and prophylactic appendectomy were performed.

The patient returned to the clinic in 1929, stating that one year previously fever of undetermined etiology had developed and had persisted for about six months; ostensibly it had subsided after some surgical dental work. Three months after cessation of the fever she suddenly had gone "to pieces"; that is, action of the heart had become rapid, the patient had become extremely nervous and irritable and again fever had developed. In the spring of 1929 "fainting spells" (extreme weakness rather than syncope) had developed; at that time her physician at home had noted that leukocytes numbered 17,000 per cubic millimeter of blood. General examination had not disclosed anything noteworthy.

The patient returned to the clinic in March 1953, because of the following: (1) pigmentation of the face, neck and hands; (2) increasing growth of hair in the areas of pigmentation of one year's duration, and (3) redness of the urine of six weeks' duration.

The pigmentation had begun as black circles under the eyes and as black, rough spots distributed around the eyes. Then diffuse pigmentation of the face, neck and hands had appeared; the appearance was such

that people inquired if the woman had just returned from Florida. Soon thereafter she had noted an isolated, rough, black spot on the ventral surface of the left forearm just below the elbow. Later she had found freckling and new hair on the dorsal surfaces of of the hands and forearms. The pigmentation and growth of hair had become progressively more intense.

When the patient noted redness of the urine six weeks before her visit of 1953 she had presumed that the color represented blood, but her home physician had assured her otherwise. The discoloration had not been constant but had appeared once or twice weekly.

As of 1953 the patient's nervousness had persisted. She had continued to have vague abdominal symptoms, which she now attributed to "colon spasms." These spasms had occurred irregularly in the left upper and left lower abdominal quadrants and had felt like "a pressure." Taking a laxative or enema had given some relief. The patient noted that her nervousness was more marked at the times when the urine was discolored. At such times she felt "shaky inside," weak, lacked her usual energy and was restless. She had not used barbiturates since her hospitalization in Rochester in 1927. At that time she had become so distraught postoperatively that the efforts of several attendants had been necessary to restrain her. She had been advised at that time never to take sedatives. She had not used alcohol until about 1943, but had taken two or three drinks two or three times weekly since that time. She believed that alcohol made her abdominal symptoms worse.

In 1938 she had been told that she was anemic and, while she did not recall the concentration of her hemoglobin, her home physician had advised her that it was at the "get busy point." She had been taking one teaspoonful of a brand* of vitamin B complex almost daily since that time.

Various other facts came out at the visit made in 1953. Beginning in 1938 and continuing intermittently until 1951, the patient had noted sudden, transient weakness of the entire left arm. There never had been complete paralysis. Her hobby was gardening and she estimated that she spent about two hours three times weekly tending her flowers; it never had occurred to her that sunlight might aggravate the pigmentation. She had been extremely constipated since her first visit to the clinic in 1927; she had not had a bowel movement in that interval without the aid of a laxative. Her hair had darkened since the onset of the pigmentation.

On physical examination at this time (1953) brownish black pigmentation of the exposed areas was seen. The melanosis affected the face, neck and the lower half of the forearms but was most intense about the temples and cheeks. Facial suffusion was distinct. The pigmentation was diffuse and freckling was superimposed. Hypertrichosis of the pigmented areas, especially noticeable on the forehead was present. On the index fingers were tiny blisters. The conjunctivae were fiery red. The blood pressure was 180 mm. of mercury systolic and 100 diastolic. The remainder of the physical examination gave results within the limits of normal.

Pertinent laboratory data included normal results of routine urinalysis, except for variations in color of the urine from yellow to brown together with a reddish tint. The concentration of hemoglobin was 16 gm. per 100 cc. of blood. The number of erythrocytes varied from 3.84 to 4.2 millions per cubic millimeter of blood. The percentage of reticulocytes varied from 1.6 to 2.5. Retention of bromsulphalein was 8 per cent in one hour. The qualitative test for urinary uroporphyrins gave a positive result and the urinary coproporphyrins were increased on two occasions. Porphobilinogen was not found in the urine on two occasions.

A follow-up inquiry did not elicit a reply.

Comment. Various examiners were impressed with the state approaching euphoria and the apparent lack of concern about cosmetic defects displayed by this patient. Numerous discrepancies in her history had been noted and more than the expected amount of confusion in carrying out instructions about collecting excreta. The question was raised as to whether or not these phenomena represented emotional features of her disease. This case was considered striking in that the abdominal symptoms and "nervousness" had been adequately noted in the records of this institution since 1927 but, that about twenty-six years passed before their ostensible basis was revealed. The acuteness of onset and the degree of pigmentation and hypertrichosis deserve emphasis. The brevity of the history of discoloration of the urine (six weeks) is surprising. In retrospect, the abdominal complaints, the nervous exhaustion, the febrile episodes, as well as the transient weakness of the left arm, may have been manifestations of porphyria.

* Betaplexin® elixir, Winthrop-Stearns, Inc.

SUMMARY AND REMARKS

Other members of the family of a patient with outspoken manifestations of porphyria may have latent porphyria. That is, they may have no symptoms or their symptoms may be so minor as to be ordinarily disregarded. Examination of the urine, however, may reveal abnormal presence of porphyrins.[22] It is possible that the absence of porphyrinuria does not exclude latent porphyria because in some patients with intermittent acute porphyria in remission it is not possible to detect abnormal porphyrins in the urine.[16] There is a real question as to the existence of so-called acquired porphyria; it may be that in such cases the condition is actually attributable to congenital errors which somehow

have been made acutely manifest. Latent porphyria is undoubtedly of more common occurrence than has been supposed.[24]

Porphyria should be considered in the presence of any obscure disturbance of the nervous system, especially unexplained peripheral neuritis, flaccid paralysis and bulbar palsy. Porphyria also should be searched for in any instance in which abdominal pain is otherwise unexplained.[21] In abdominal crises to which patients with porphyria are subject, it may be very difficult to exclude the diagnosis of obstruction of the large bowel.

In the presence of the congenital type of porphyria aspirated bone marrow may contain porphyrins in large amounts. The same may be true of liver obtained at biopsy in the intermittent acute or chronic type (known together as "porphyria hepatica"). Procedures for making these determinations probably are of value, however, only in institutions especially devoted to research.

The appearance of freshly voided urine varies widely, especially in the presence of the acute and chronic types of porphyria. It is customary to think of porphyric urine as being red or of port-wine color but this is by no means the general rule. In some cases the specimen is of almost normal color, varying from light yellow to dark amber. Not all the discoloration of porphyric urine is due to porphyrins. Porphobilin is responsible in large measure for discoloration of the urine in some cases of porphyria hepatica.[16] The urine contains other pigments which are not porphyrins but which probably are related to the disturbance in pyrrole metabolism.[23] Uroporphyrins, as well as coproporphyrins, may appear in the urine as precursors or chromogens. Large amounts of the compounds may be overlooked unless steps are taken to convert the chromogens; the chromogens of uroporphyrin are readily converted by heating.

In 1953, at the clinic, eight additional patients were seen who excreted only porphobilinogen in the urine but examination of whom otherwise presented only equivocal evidence of porphyria. This finding has left us in some doubt as to the normality of the pyrrole metabolism of these patients. We have tentatively considered them to have demonstrated a so-called falsely positive reaction for porphobilinogen. However, these patients may ultimately be proved to have porphyria. That this is possible became apparent on reviewing the record of a patient with porphyria in whom necropsy revealed large deposits of porphyrins in the bones, yet only porphobilinogen had been excreted in the urine except for a single excretion of uroporphyrin just before death. One other patient who excreted only porphobilinogen initially later excreted uroporphyrin and excessive amounts of coproporphyrin. A third patient initially excreted excessive amounts of coproporphyrin, uroporphyrin and porphobilinogen but on later examination excreted only porphobilinogen. We have considered that three of our patients have porphyria, although to date they have excreted only porphobilinogen.

Watson[24] recently discussed the occurrence of porphobilinogenuria in (1) liver disease, (2) malignant disease and (3) infectious or nervous disease. He thought it not clear whether persons who had these diseases had latent porphyria or whether the porphobilinogenuria was strictly secondary. He re-emphasized that there occur certain cases, best classified as instances of porphyria, which are characterized by porphobilinogenuria with little or no porphyrinuria.

The simplicity of the Ehrlich reaction employed to screen urines with respect to the diagnosis of porphyria hepatica, and especially of the intermittent acute type, warrants emphasis. Porphobilinogen gives an Ehrlich reaction similar to that of urobilinogen, but porphobilinogen is not extracted from the urine by ether, as is urobilinogen. In addition, the Ehrlich aldehyde compound of porphobilinogen is insoluble in chloroform, while the urobilinogen aldehyde is readily soluble. The simplicity of the test makes it a readily available office procedure.

No treatment will rid the victim of porphyria of his disease. Nevertheless, those patients who have the disease and who are photosensitive should, of course, be restrained from exposing themselves to the sun and to ultraviolet light. Splenectomy may be attempted in the congenital type if severe hemolytic anemia occurs. Bleaching agents may be tried for the patient who becomes hyperpigmented. A diet high in carbohydrate and proteins should be recommended for patients with acute or chronic porphyria (porphyria hepatica). For them, barbiturates and alcohol are strongly interdicted; the propensity of these agents to induce crises is corroborated by their ability to increase excretion of porphyrin. Ganglion-blocking agents such as tetraethylammonium chloride may have application in the treatment of patients with

abdominal pain. Chlorpromazine (thorazine) has been used with good results in some patients with abdominal pain. The use of adrenocorticotrophic hormone (ACTH) and allied agents in the management of porphyric "crises" is not yet completely established. Opiates, meperidine hydrochloride (demerol hydrochloride), methadone hydrochloride (adanon® hydrochloride), paraldehyde and chloral hydrate apparently do not precipitate or aggravate attacks.

REFERENCES

1. Schwartz, S. Clinical aspects of porphyrin metabolism. *Veterans Admin. Tech. Bull. TB* 10–94, 6: 1, 1953.
2. Watson, C. J. Porphyrin Metabolism. In: Duncan, G. G. Diseases of Metabolism: Detailed Methods of Diagnosis and Treatment, 3rd ed., pp. 1079–1103. Philadelphia, 1952. W. B. Saunders Co.
3. Gray, C. H., Rimington, C. and Thompson, S. Case of chronic porphyria associated with recurrent jaundice. *Quart. J. Med.*, 17: 123, 1948.
4. MacGregor, A. G., Nicholas, R. E. H. and Rimington, C. Porphyria cutanea tarda: investigation of case, including isolation of some hitherto undescribed porphyrins. *Arch. Int. Med.*, 90: 483, 1952.
5. Günther, H. Die Hämatoporphyrie. *Deutsches Arch. klin. Med.*, 105: 89, 1912.
6. Günther, H. Hämatoporphyrie. In: Enzyklopaedie der klinischen Medizin, vol. 2. Handbuch der Krankheiten des Blutes und der blutbildenden Organe: Haemophilie, Haemoglobinurie, Haematoporphyrie, pp. 622–673. Berlin, 1925. Julius Springer.
7. Hoppe-Seyler. Cited by Günther, H.[5,6]
8. Fischer, H. Über das Urinoporphyrin. I. Mitteilung. *Ztschr. f. physiol. Chem.*, 95: 34, 1915.
9. Fischer, H. Über das Kotporphyrin. II. Mitteilung über das Urinporphyrin. *Ztschr. f. physiol. Chem.*, 96: 148, 1915.
10. Fischer, H., Hilmer, H., Lindner, F. and Pützer, B. Zur Kenntnis der natürlichen Porphyrine. XVIII. Mitteilung. Chemische Befunde bei einem Fall von Porphyrinurie (Petry). *Ztschr. f. physiol. Chem.*, 150: 44, 1925.
11. Schwartz, S. and Watson, C. J. Isolation of uroporphyrin from the feces in idiopathic porphyria. *Proc. Soc. Exper. Biol. & Med.*, 47: 390, 1941.
12. Fischer, H. and Zeile, K. Synthese des Hämatoporphyrins, Protoporphyrins und Hämins. *Ann. d. Chem.*, 468: 98, 1929.
13. van den Bergh, A. A. H. and Hyman, A. J. Studien über Porphyrin. *Deutsche med. Wchnschr.*, 54: 1492, 1928.
14. Schwartz, S., Glickman, M., Hunter, R. and Wallace, J. The Relation of Erythropoiesis to the Excretion of Coproporphyrin by Dogs and Rabbits, and to the Concentration of Coproporphyrin and Protoporphyrin in Rabbit Erythrocytes. Argonne National Laboratory, C. H. 3720, 1947.
15. Watson, C. J., Hawkinson, V., Schwartz, S. and Sutherland, D. Studies of coproporphyrin. I. The per diem excretion and isomer distribution of coproporphyrin in normal human urine. *J. Clin. Investigation*, 28: 447, 1949.
16. Lowry, P. T., Schmid, R., Hawkinson, V. E., Schwartz, S. and Watson, C. J. Porphyria: clinical manifestations in relation to chemical findings. *Bull. Univ. Minnesota Hosp. & Minnesota M. Found.*, 22: 97, 1950.
17. Waldenström, J. Some observations on acute porphyria and other conditions with a change in the excretion of porphyrins. *Acta med. Scandinav.*, 83: 281, 1934.
18. Waldenström, J. Untersuchungen über Harnfarbstoffe, hauptsächlich Porphyrine, mittels der chromatograpischen Analyse. *Deutsches Arch. klin. Med.*, 178: 38, 1935.
19. Waldenström, J. Studien über Porphyrie. *Acta med. Scandinav.* (suppl. 82), p. 1, 1937.
20. Emerson, K., Jr. Disorders of Pigment Metabolism. In: Harrison, T. R. Principles of Internal Medicine, pp. 717–723. Philadelphia, 1950. Blakiston Co.
21. Watson, C. J. Porphyria. In: Cecil, R. L. and Loeb, R. F. A Textbook of Medicine, 8th ed., pp. 662–666. Philadelphia, 1951. W. B. Saunders Co.
22. Brunsting, L. A. and Mason, H. L. Porphyria with cutaneous manifestations. *Arch. Dermat. & Syph.*, 60: 66, 1949.
23. Brunsting, L. A., Mason, H. L. and Aldrich, R. A. Adult form of chronic porphyria with cutaneous manifestations: report of seventeen additional cases. *J. A. M. A.*, 146: 1207, 1951.
24. Watson, C. J. Some studies of nature and clinical significance of porphobilinogen. *Arch. Int. Med.*, 93: 643, 1954.

Seminar on Allergy

Types and Distribution of Antibodies*

WILLIAM J. KUHNS, M.D.†
Pittsburgh, Pennsylvania

THE purpose of this seminar is to provide a review of investigations which have been carried out on the nature of circulating antibodies, including circulating allergic antibodies. Cellular and tissue antibodies will not be discussed. For further related topics the reader is referred to articles by Boyd,[1] Haurowitz,[2] Smith and Jager,[3] Grabar,[4] Mayer,[5] Campbell,[6] Treffers,[7-8] and Kabat.[9]

Antibodies present in blood may be best demonstrated when, under a given set of laboratory conditions, they interact with their corresponding determinants. A method which indicates that a specific antigen-antibody union has occurred is required to provide evidence for the existence of antibodies. Antibody distribution in components of serum or plasma is usually determined after a specific method for demonstrating antibody in whole serum has been applied. Then partition or separation of serum or plasma components, based on a knowledge of certain chemical or physical characteristics, may be carried out and the separated materials are individually available for testing again by means of specific immunologic or immunochemical methods. Discrimination as to the choice of partition technic may at times be quite important when one is concerned about the preservation of labile properties associated with a given antibody.

METHODS USED FOR DETECTION OF ANTIBODIES

Antigen-antibody interactions may result in different phenomena. These interactions may depend upon the following factors: the soluble or particulate nature of the antigen, toxicity or enzyme activity associated with the antigen, the presence of an associated biological property of the antigen, or the availability of one or more than one combining sites on the antibody. Consequently, the end result of a reaction between antigen and antibody may be precipitation, agglutination, lysis, complement fixation and hemolysis, toxin or enzyme neutralization, immediate wheal reactions in human skin or inhibition of these effects, or anaphylaxis or an Arthus reaction.

It was originally thought that different technics for measuring antibody detected different kinds of antibody. For example, an agglutinin and a precipitin were regarded as distinct antibodies. However, it has since been found that more than one technic may indicate the presence of only one kind of antibody, although titers of a given antibody may not be the same when tested by two methods because of differences in sensitivity between methods; thus, a precipitating antibody may also be agglutinating antibody. Quantitative confirmation of this circumstance was provided by Heidelberger and Kendall[10] and Heidelberger and Kabat.[11]

The destruction of certain bacteria by phagocytes is greatly increased following the addition of immune serum to the leukocytes. Substances in serum which are able to produce this effect are termed opsonins. The ability of opsonins to promote phagocytosis of their homologous antigens by white cells provides the basis for a laboratory test (opsonophagocytic index).

* From the Central Blood Bank of Pittsburgh and the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

† The author's own investigations described in this article have been carried out at the following institutions: New York University College of Medicine, Department of Microbiology, 1950 to 1951; Hospital of the Rockefeller Institute for Medical Research 1951 to 1954, and the University of Pittsburgh School of Medicine 1954 to the present. Financial support was derived from the following organizations: United States Public Health Service 1950 to 1951; Life Insurance Medical Fund 1951 to 1952; Helen Hay Whitney Foundation 1951 to 1952, and American Heart Association 1952 to 1955.

Each animal species forms its own protein independent of the protein ingested. Another antibody detection method is based upon this fact. A human antibody, or human gamma globulin, may function as antigen in another species and antibody to it can be formed in the animal. When serum is removed from the animal and is mixed with the proper amounts of human gamma globulin, a precipitate is formed. This test may be used clinically, as in the disease agammaglobulinemia. No precipitate forms if sera from these patients are mixed with anti-antibody serum.

The amount of antibody in a serum may be demonstrated in several ways. The simplest technic is to make serial dilutions of antiserum to each of which is added a given amount of antigen. An end point or titer is indicated by the last dilution in which agglutination occurs in the case of a particulate antigen or in which precipitation occurs with soluble antigens. Wu[12] and Heidelberger[10] have developed quantitative technics for the measurement of precipitable or agglutinable antibody. Another method to visualize interactions between antigen and antibody is precipitation in a semi-solid medium, generally agar or gelatin.[13–15] This test is capable of showing the presence of more than one antigen-antibody system. A visible and discrete opaque band which migrates through the agar or gelatin is indicative of the reaction between components of at least one system. The presence of more than one band indicates the presence of more than one antigen-antibody system.

Information about the reactivity of an antibody may be obtained by determining the rapidity with which antibody reacts with antigen as judged by precipitation, agglutination or lysis. Lack of aggregation may indicate an unusual antibody, and further special confirmatory tests may be required. As an example, a suspected non-aggregating antibody may inhibit the interaction between aggregating antiboby and its antigen.

THE ROLE OF ANTIGENS IN DETERMINING NATURE AND SPECIFICITY OF ANTIBODIES

An antigen, by definition, is a material capable of stimulating antibody formation in a host. The criteria for antigenicity are few. The material in question must not possess too simple a structure, although even molecules of low molecular weight can at times be antigenic (haptens) when they are conjugated with higher weight materials. Generally, a substance which is antigenic is foreign to the tissues of the host; but studies of the lens,[16,17] testis[18,19] and brain[20,21] tissues injected in homologous species suggest that this is not necessarily a prerequisite.

Studies have indicated that the antibody response may be influenced by chemical differences between antigens. For example, Landsteiner[22] demonstrated that a titer of antibodies formed against a single haptenic substance could be partially reduced by absorption with materials which were chemically similar but altered perhaps only with respect to the location of one methyl group on a benzene ring.[23,24] When he treated antigens with diazotized amines the presence of strongly acidic or basic groups markedly influenced the specificity of antibodies produced against them.[22]

In the case of antisera formed against native serum proteins, a cross reaction with heterologous serum proteins may occur because an antigen contains similar groups capable of reacting with more than one antibody; also, the antibody molecules can differ in degree or rate of reactivity. Hooker and Boyd[25] found that crystalline duck egg albumin will precipitate a portion of the antibody to antiserum formed against crystalline hen egg albumin. Cross reacting antibodies against pneumococcal types III and VIII polysaccharides have also been demonstrated by Heidelberger, Kabat and Shrivastava.[26] Upon further study of this reaction it was found that both antigens possessed the same structural unit, that of cellobiuronic acid.[27] A serologic relationship between anthrax carbohydrate, pneumococcus type XIV and blood group substance[28–31] has also been demonstrated to be due to chemical similarities between the polysaccharides contained in these substances. Cross reactivity exists in a number of diverse systems, including mammalian thyroglobulins,[32] bacterial systems[33] and plant viruses.[34] The problems posed by cross reactivity with respect to relating chemical structure and immunologic specificity have been discussed by Kabat.[35]

The chemical purity of an antigen may have considerable bearing on the antibody response. Trace impurities in an antigen may elicit the formation of immune bodies in the same manner as the major antigenic constituent. The fact that a multiple response has occurred may or may not be detected by immunochemical

methods. Observations on the significance of trace materials in allergies have been provided by Vaughan and Kabat[36] who immunized rabbits with recrystallized egg albumin preparations. The antiserum produced contained antibodies not only against ovalbumin, but also conalbumin, ovomucoid and lysozyme. By means of absorption technics and passive transfer studies of absorbed sera in human subjects it was shown that wheal and erythema activity was caused by interaction between antigens and antibodies which were unrelated to the four main antigen-antibody systems. Thus antibodies possessing a marked biologic effect could be produced against infinitesimally small amounts of contaminants in an antigenic preparation.

The antibody response may also be influenced by the physical state of antigens. The admixture of antigen with an adjuvant medium followed by injection into a suitable host usually causes enhanced antibody production in the host.[37]

In the field of allergy the nature of the antigen is important as related to its ability to cause immediate wheal reactions in skin when combined with antibodies. Repeated injections of large amounts of grass and ragweed pollens cause little or no production of skin-sensitizing antibodies[38] as judged by the development of skin reactivity to the pollen preparations, whereas extracts of Ascaris produce skin-sensitizing antibodies in a majority of persons given subcutaneous injections.[39]

Another factor which may influence the kind of antibody produced upon stimulation with antigen is the mode of injection into the host. Treffers, Heidelberger and Freund[40,41] have shown that horse antibodies formed against rabbit serum albumin and globulin may be "univalent" or incomplete, or they may be largely multivalent depending upon the route of inoculation used—intradermal or intravenous—and whether or not albumin or globulin were used as antigens. Grabar[4] quotes Bussard as having observed differences in the quantity and quality of precipitating and non-precipitating antibodies against chorionic gonadotrophin, depending upon whether or not the rabbits were splenectomized and whether or not the route of the injections was intravenous or subcutaneous.

Repeated immunization with antigens will affect the formation of immune bodies, both qualitatively and quantitatively. Serial observations on sera taken from horses hyperimmunized with diphtheria toxoid have indicated that as the number of injections increases over a period of time a rise in antibody titer will usually occur. Furthermore, changes occur in the flocculating characteristics of the antibodies produced and also in the electrophoretic distribution of these antibodies.[42,43] Since the immune response of horses to purified toxoid may include production of antibodies other than antitoxin, these findings may be influenced by the presence of qualitatively different antibodies which could differ from one another in regard to their electrophoretic distribution.[44] It is of interest that the qualitative changes in horse antitoxin observed following prolonged immunization with diphtheria toxoid are not present in sera from horses immunized with pneumococcal polysaccharides.[27]

IMMUNOLOGIC BEHAVIOR OF ANTIBODIES

1. "*Natural*" *Antibodies.* Antibodies may occur in human beings or animals who have had no known contact with the corresponding antigen or immunizing agent. Such antibodies may result from unapparent mild infections with a given organism or from invasion by antigenically related strains. The naturally occurring isohemagglutinins in man have been stated to be the result of immunization by antigens related to blood group A and B or other substances,[45] of which many exist in nature; however, complete agreement does not exist on this point.[46] Normal hemagglutinins and hemolysins acting on red blood cells of foreign species and plant agglutinins or phytoagglutinins[22,47,48] are further examples of so-called natural antibodies. It has been suggested that the name "lectin"[1] be applied to materials such as phytoagglutinins which may react as antibodies because of chemical similarities to the combining groups of their antigens.

2. *Natural Substances Participating in Immune Phenomena. Complement:* Complement is a bactericidal substance present in normal sera.[49,50,51] In contradistinction to antibodies which are induced by immunization procedures, it is unusually susceptible to the effects of heating at 56°C. or aging.[52] Complement is not a single serum constituent but a complex of several materials, including polysaccharide-containing proteins, and is present in more than one globulin fraction.

Four factors of complement (C′ 1, 2, 3, 4) are recognized and the titer of whole complement is a function of the component present in lowest titer. The hemolytic activity of complement depends upon the presence in serum of a separate hemolysin. This consideration, along

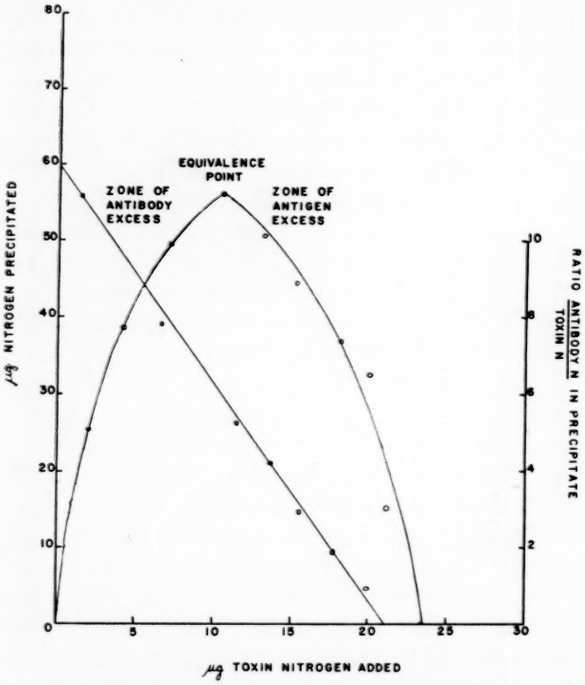


FIG. 1. Quantitative toxin–antitoxin precipitation reaction. The antitoxic serum used was from a normal human subject in whom a high antitoxin titer was formed following a single booster dose of diphtheria toxoid. Each point on the rounded curve represents nitrogen in a precipitate which formed after toxin had been added to a 1 to 6 dilution of serum. Antitoxin N is calculated by subtracting toxin N from total N present in the washed digested precipitate.

with others such as salt concentration, pH, and magnesium and calcium ions, is of importance in laboratory procedures used to determine complement activity.[27]

Properdin: Recently a new natural immune substance, properdin, has been described by Pillemer and his associates.[53] It was isolated because of its ability to form an insoluble complex with yeast zymosan at 17°C. In the presence of a component of complement (C′ 3) and magnesium ions, properdin participates in bactericidal, virus-neutralizing and hemolytic reactions. However, it is not a component of hemolytic complement and it is not necessary in specific antigen-antibody combinations. Pillemer[54] has made an interesting observation on the possible relationship of properdin to natural immunity. He found that most substances which combine with or inactivate properdin possess infection-promoting activity. They include complex sugars such as dextrans and levans, hog gastric mucin, bacterial cell walls and other bacterial carbohydrates. This suggests that properdin could act as a sort of buffer if exposed to infecting agents possessing a certain chemical composition. Furthermore, Pillemer suggests that the interaction of bacterial endotoxins with properdin may provide a basis for "tolerance" to the endotoxin in the Shwartzman reaction and pyrogenic or other manifestations of endotoxins.

3. *Detection of Antibodies Produced by Immunization. Precipitation:* The specific interaction between a soluble antigen and its antibody may be followed by appearance of a precipitate, an insoluble antigen-antibody combination. Antibodies capable of eliciting this phenomenon are termed precipitins. The amount of precipitate formed will depend upon the absolute amounts of antigen and antibody present and the ratio in which they are mixed.

The precipitation reaction may be quantitated by methods developed by Heidelberger.[10] In this procedure different amounts of antigen are added to similar aliquots of a precipitating antiserum distributed in several tubes. A precipitate may form in each tube, and there will be an equivalence point or zone where the maximal amount of antibody is carried down with its antigen as a precipitate. This is illustrated in Figure 1. The precipitates may be quantitated by means of ultraviolet absorption spectroscopy,[55] colorimetry employing the Folin-Ciocalteu phenol reagent,[56] or analysis of precipitated nitrogen using modifications of the Kjeldahl method.[57,58]

The ratio of antigen to antibody nitrogen determined at the point of equivalence will vary depending upon the system used.[27] Quantitative immunochemical studies have been carried out in many systems, including diphtheria toxin and antitoxin,[59,60] pneumococcal polysaccharides and their antisera,[61,62] and components of egg white and their antisera.[63] The configuration of the curve which represents precipitated nitrogen may depend upon the antigen and antibody employed, the species of the animal receiving the antigen, the presence of denatured antigen or antibody, or the presence of impurities in antigenic preparations.

For most protein antigens and their antibodies there is a sharp equivalence point and a rapid decrease in precipitable nitrogen with an excess of either antibody or antigen. (See Figure 1.) Tests of supernatant fluid in antibody excess demonstrate the presence only of antibody; in antigen excess, only antigen is present. No antigen or antibody is present in supernates obtained at the point of equivalence. Exceptions to the usual kind of precipitation curve are provided by the reactions of pneumococcal polysaccharides and antipneumococcal antibodies,[61,62] dextrans and their antibodies,[64] certain flocculating antibodies occurring in the horse,[59] and physically or chemically altered antigens or antibodies;[5,60] in these instances, maximum precipitation occurs over a wide zone of antigen addition.

If more than one antigen and one antibody occur together, one or more of the following differences from the immunochemical values which characterize single systems would be observed: (1) There may be a prolongation of the precipitation curve in antigen excess, or more than one peak on the curve; (2) examination of supernatants in a precipitin reaction may reveal the concomitant presence of antigen and antibody; (3) precipitation as bands may be observed when the materials interact in a semi-solid medium. Sera which contain antibodies against an antigen and its impurities may be absorbed only with the contaminating antigens. If precipitation tests or supernatant tests are carried out on the absorbed sera, the loss of contaminating antibodies as the result of absorption may be indicated by changes in the tests described.

The reaction between diphtheria toxin and horse antitoxin differs from the usual precipitin reaction in that the specific precipitate is soluble in excess of either antibody or antigen.[65] In addition, equivalence between both substances occurs over a broad zone rather than at a sharp point, as is observed in other precipitin reactions. The phenomenon is referred to as "*flocculation*" and the curve depicting it is referred to as a "flocculation-type curve." Horse antisera produced against scarlatinal toxin,[66] hemacyanine[67] and egg albumin[68] may react similarly. This property appears to be peculiar to the horse, since it has never been demonstrated in sera from other species. Pope and Stevens[69] have been able to change the kind of curve shown by some horse antitoxins from the flocculating to the precipitating variety. This could be demonstrated following the removal of non-antitoxic antibodies by absorption technics.

"*Univalent*" *Antibodies.* In studying quantitative precipitin reactions in the egg albumin system Heidelberger and Kendall[63] obtained immunochemical evidence that not all antibodies formed against an antigen react to form a visible precipitate. However, antibodies which did not themselves form a precipitate could add onto a mixture of the specific antibody and its antigen (coprecipitation). The term "univalent" was applied to these antibodies because it was thought that each molecule possessed only a single immunologically reactive group, in contrast to precipitating or multivalent antibodies which contain more than one combining group. Pappenheimer[68] studied consecutive specimens of serum from a horse which had been immunized with egg albumin and found that serum from an early bleeding contained only "univalent" antibody, but that later bleedings from the same horse contained precipitating antibody which upon quantitation showed the antitoxin type of curve. Further reference will be made later to the possible role of "univalent" antibodies in allergies.

Danysz[70] found that whether diphtheria toxin was added to antitoxin fractionally or all at one time was of importance in relation to the kind of complex formed between both reagents (*Danysz effect*). He was able to demonstrate that far less toxin was neutralized by a given amount of precipitating antitoxin if the total amount of toxin was added in successive portions than if the same amount was added in a single portion. Two sets of experiments employing the same total quantities of antigen and antibody are required and, because the antigen in this instance is toxic, guinea pigs weighing about 250 gm. are used to test the toxicity of mixtures. The results of two typical experiments are as follows: (1) Guinea pigs receiving sufficient amounts of mixtures of antitoxin and equivalent toxin added in two successive portions die in four days or less; (2) mixtures of antitoxin and equivalent toxin added at once are not lethal in four days.

This phenomenon occurs because toxin and antitoxin may combine in more than one proportion depending upon their relative concentrations. If only a small amount of toxin is added to a relatively large amount of antitoxin, the principal product will possess the molecular

composition TA_4 and this will aggregate to a soluble polymer of high antitoxin content. Since the latter aggregation is only slowly reversible, the rest of the toxin, when added, will combine with whatever free antitoxin is left to form complexes of low antitoxin content which are in equilibrium with free toxin. If, instead of adding toxin in portions, one adds a single amount of toxin to antitoxin at the flocculation point, the average molecular composition of floccules which settles out is TA_2.[59]

A Danysz effect would not be expected to occur if antigen were mixed with antibody which possessed only one reactive site, that is, univalent antibody. It has been found that non-precipitating diphtheria antitoxin does not cause a Danysz effect.[71]

Agglutination: When bacteria, red blood cells or other particulate agents are specifically clumped in the presence of their corresponding antisera, the reaction is termed agglutination. The antibodies which produce this effect are called agglutinins. The primary difference between agglutination and precipitation is related to the size of the antigen which determines whether agglutination or precipitation occurs. If antigen particles are large enough to be sedimented by low speed centrifugation, or if they appear as discrete particles under the microscope, the aggregation of the suspended particles is properly described as agglutination.[27] It was stated previously that an agglutinin may also be a precipitin. Antibodies formed in sufficient titer against pneumococccal polysaccharides are precipitated by the specific soluble substance; however, the same antibodies will cause agglutination of pneumococci containing this substance.

The heterogeneous nature of relatively large antigenic structures which can be agglutinated, e. g., bacteria, may cause difficulties in deciding whether their agglutination by a serum is due to a single antibody. Qualitative end point titers carried out on such sera may give no indication of the presence of multiple antigen-antibody systems but simply reflect the presence of the antibody present in greatest amount. This inherent drawback limits the possibility of strict quantitation. Nevertheless, the value of agglutination as a simple technic for identifying and measuring antibodies has been demonstrated in many phases of diagnostic medicine.

If red cells are treated with appropriate chemicals or serologic reagents they may be agglutinated by antibodies or other materials which would otherwise have no effect on them. Erythrocytes treated with tannic acid combine with some soluble protein antigens, including diphtheria toxoid[72] and allergic extracts;[73] this provides an agglutination test for antitoxins and allergic antibodies. Normal sera contain a substance which agglutinates periodate-treated red cells.[74,75] Antibodies formed in tuberculosis may agglutinate red cells exposed to tuberculin.[76] When the red blood cells of the sheep, goat or cow have been exposed to sheep amboceptor they are rendered agglutinable by a factor contained in sera of patients with rheumatoid arthritis.[77] Sensitized sheep cells are also agglutinated by heterophile antibodies from patients with infectious mononucleosis.[78] These are but a few of the many modified hemagglutination technics now in use.

Complement fixation and lysis: Immune hemolysins or amboceptors are antibodies which will lyse sensitized red cells in the presence of complement. Cells are said to be sensitized when these antibodies, prepared by immunizing rabbits with sheep red cells, have been allowed to interact with them. Persons who possess A or B blood group isohemagglutinins may possess hemolysins for the corresponding blood groups.[79] In some disease states abnormal hemolysins are present which are active for red cells only at cold or warm temperatures.[80] A cold hemolysin is responsible for the Donath-Landsteiner reaction in paroxysmal cold hemoglobulinuria.[81] A hemolysin in normal serum lyses the abnormal red cells of patients with paroxysmal nocturnal hemoglobulinuria at acid pH.[82] Other hemolysins are occasionally demonstrated in patients with acquired hemolytic anemia.[80] Lysins for bacteria may be demonstrated by exposing specific antiserum to the bacteria in the presence of complement.

Complement may be used as an indicator for antigen-antibody reactions because it can combine with specific complexes. The chemical basis of this reaction is unknown, but such factors as the relative amounts of each reagent in mixture, the kind of antigen and antibody, and the particle size of aggregates influence the extent to which complement is bound.[83] Complement fixation may be demonstrated by adding sensitized red cells to a mixture of antigen, antibody and complement. Fixation of complement is indicated by a lack of hemolysis of the sensitized cells. In the absence of antigen or antibody, or

in the presence of complexes which do not fix complement, the sensitized red cells combine with complement and then become hemolyzed. Antigen-antibody systems differ in their ability to fix complement. Usually fixation occurs in the presence of precipitating antibodies and their antigens but horse antisera to pneumococcus type specific polysaccharides do not fix complement. Some non-precipitating antibodies including skin sensitizing diphtheria antitoxin and other allergic reagins[71] fix complement poorly or not at all.

Protective antibodies and neutralizing antibodies: Since antibodies interfere with some biologic actions of antigens, it is possible to quantitate antibody activity in sera by demonstrating the degree to which such interference is produced. Antisera formed in immunized animals may protect against a lethal dose of toxin if passively injected at the correct time. Titers of antitoxin may be measured by injecting varying proportions of toxin and antiserum into animals and observing the reaction. This is known as a protection test and the kind of antibodies participating in the reaction are called protective antibodies. They may or may not possess immunologic functions other than that related to their protective ability. Protection tests are used to demonstrate antibodies formed against a number of bacterial antigens, viruses and rickettsia. If additional tests using these antigens are available, it may be possible to derive information about the nature of the antibodies. Thus complement-fixing antibodies as well as protective antibodies are formed in yellow fever.[84] This could represent the presence of antibodies formed against different viral antigens or qualitatively different antibodies formed against the same antigen. The combination of an *in vivo* and *in vitro* method for detecting antibody may be of specific use in demonstrating qualitative antibody differences. This is illustrated by the following example. No correlation between the mouse protective power of fractionated horse pneumococcal antisera and their precipitating antibody content has been obtained.[85] However, when protection tests and precipitation methods were carried out using whole serum instead of purified reagents parallel titers were demonstrated by both technics.[86]

Some bacterial organisms produce materials which are directly toxic to a susceptible host, giving rise to characteristic symptoms or lesions, or to death. Toxins may be secretory products of bacteria (exotoxins) or a structural component of the organism itself (endotoxins). A great number of toxins also function as antigens. It is therefore possible for a host to form antitoxins in sufficiently high titer to combine with and neutralize the biologic activity of certain toxic agents. The reaction of rabbits and of guinea pigs to mixtures containing diphtheria toxin and antitoxin are used to titrate the antitoxin.[87] Mixtures containing tetanus antitoxin are generally assayed in guinea pigs. Toxins may also function as hemolysins, leukocidins, fibrinolysins, hyaluronidases or proteolytic enzymes, and biologic assays for antibody activity against them may be based on some of these properties.[88] Thus antibodies produced against streptococci or their products may be tested by means of an indicator system composed of the specific substrate of the antigen in question.[89] For instance, antibodies against streptococcal fibrinolysin are measured in the presence of a clotting system. Antibodies formed against such materials may or may not function as precipitins, agglutinins, and the like. Streptococcal antihyaluronidase is not agglutinable or precipitable. On the other hand, a potent precipitating antitoxin may be produced against scarlet fever or erythrogenic toxin.[66]

The biologic or catalytic activity of a protein does not necessarily parallel its antigenic properties. This principle is well illustrated by the behavior of enzymes which for many years were considered to be non-antigenic. Recently it has been found that many enzymes are, in fact, antigenic but the antibodies produced against them do not always neutralize the activity of the enzyme. Thus the enzyme tyrosinase is completely precipitated by antibody formed in the rabbit and human but the enzymic activity is unaffected by precipitation with antibody.[90] There are other similar examples. Urease-antiurease floccules retain most of the urease activity in the floccules[91] and catalase is practically unimpaired when mixed with anti-catalase.[92] On the other hand, antibodies formed against three streptococcal products, streptokinase, desoxyribonuclease and hyaluronidase, neutralize the respective biologic actions of these products. Indeed, the only reliable indicator of antibody activity is provided by this effect since the antibodies in these instances cannot be effectively titrated on the basis of agglutinating or precipitating activity.[89]

Skin-sensitizing antibodies: Prausnitz and Küstner in 1921[93] first demonstrated a serum factor in allergic subjects which upon passive transfer could cause local wheal and erythema reactions in normal human skin if it was in contact with its corresponding allergen. The reaction was found to be highly specific but the antibody causing it differed from most antibodies in the following respects: (1) It was not precipitable by its antigen; (2) it did not fix complement; (3) it was incapable of sensitizing guinea pigs to anaphylactic shock; (4) its activity was greatly impaired by heating at 56°C. for a half hour, and (5) local passive sensitization produced by this antibody persisted for weeks. The name atopic reagin[94] or skin-sensitizing antibody was applied to this substance.

Qualitative end point titers may be determined on reaginic sera, and the wheal and erythema reaction produced when reagin is passively transferred and challenged with antigen serves as a means of designating end points. Another method of titration developed by Lippard and Schmidt[95] is to introduce mixtures of dilutions of antigen containing constant amounts of reaginic serum into skin sites. Subsequent challenge with antigen one to three days later is made either intramuscularly or intradermally at each site. Wheal reactions are caused at the sites which contain reagin in excess. This is known as the neutralization technic.

High titers of skin-sensitizing antibodies contained in human allergic sera generally cannot be precipitated but evidence exists that they may be coprecipitated when added to related antigen-antibody precipitates.[71] In the rabbit skin-sensitizing antibodies formed against trace antigens in egg white can be precipitated, and they can also be passively transferred to human skin.[36] Subjects who are immunized with dextran develop cutaneous sensitivity associated with the formation of precipitins.[64,96] Subjects who do not form precipitins do not develop cutaneous sensitivity. However, sensitivity, when produced, cannot be passively transferred to skin of normal human subjects by means of antidextran antisera.

Studies using diphtheria toxoid as an immunizing antigen indicate that in the secondary response to toxoid a non-precipitating antitoxin may be formed which upon passive transfer into human subjects[97] possesses wheal and erythema producing properties. For the purpose of carrying out various immunochemical investigations diphtheria toxin and toxoid possess certain advantages over other more complex antigens such as pollens. Both reagents are available in a high state of purity and are reasonably well characterized proteins. Several sensitive tests are available for the detection of toxin and antitoxin even when present in low concentrations. Toxoid is a convenient substance to use because one dose in a subject who gives a negative reaction to the Schick test is usually followed by a rapid and high antitoxin response consisting of precipitating and/or non-precipitating antitoxin. Non-precipitating antitoxin may occur in two forms: (1) skin sensitizing, and (2) non-skin sensitizing. It is possible to quantitate skin-sensitizing antitoxin in human subjects who give a positive reaction to the Schick test because of its ability to remain at injection sites, a property which enables this antibody to convert discrete sites on an appropriate subject to areas which give negative results to the Schick test. The test can be quantitated quite accurately and depends upon the ability of this kind of antitoxin to neutralize Schick toxin introduced later at the same site. After passive transfer of appropriate dilutions of an antitoxin the inhibition of delayed reactions to toxin would indicate the presence of skin-sensitizing antitoxin.

During immunization of human subjects with diphtheria toxoid high titers of skin-sensitizing antitoxin may develop without the occurrence of clinical allergy, although it was noted that subjects who gave a history of long standing allergies were more likely to possess immediate reactivity upon testing with intradermal toxoid than a person who gave no such history.[97] Persons who are ill with hay fever or asthma seem to be more readily disposed to develop skin-sensitizing activity, possibly for constitutional or hereditary reasons. However, Lowell[98] has indicated that hay fever and asthma are not common among the relatively few patients who develop high degrees of allergy and skin reactivity while under treatment with insulin or liver extracts. The development of allergies under these circumstances is entirely unpredictable.

Anaphylaxis: Anaphylaxis, an *in vivo* manifestation of combination between antigen and antibody, shows the same degree of specificity as do other kinds of antigen-antibody reactions. Active anaphylaxis can provide little informa-

tion about the quantitative or qualitative nature of antibodies. It may be of value in establishing whether a material is antigenic or whether there are antigenic impurities in a preparation. If injection of certain immune sera into normal guinea pigs is followed by injection of antigen several days later, a typical anaphylactic response may occur. This phenomenon is called passive anaphylaxis. Quantitative studies of this reaction by Kabat[99,100] have provided a more accurate understanding of the importance of the amounts of antigen and antibody required and of the time interval between injections of antigen and antibody. For example, as little as 0.03 mg. of rabbit anti-ovalbumin nitrogen sensitized guinea pigs so that the subsequent injection of 0.16 mg. of ovalbumin caused fatal anaphylaxis. The non-precipitable or "univalent" antibody remaining after serial absorption of rabbit anti-ovalbumin sera was found to be as effective in causing passive anaphylaxis as precipitating antibody.[101] Similar studies employing diphtheria toxoid and antitoxin indicated that non-precipitating skin-sensitizing antitoxin was also as effective as precipitating antitoxin in causing systemic anaphylaxis.[71,99] However, Kabat[102] and other workers[103] have shown that severe Arthus reactions can be produced passively in the guinea pig only by precipitating antibodies.

Inhibiting or blocking antibodies: Combination between antigens and antibodies may occur with or without visible evidence of agglutination, precipitation, lysis, or wheal and erythema reactions. The absence of some of these reactions may mean that insufficient amounts of antigen or antibody are present or that the antibodies combine very slowly with their antigens. On the other hand, antibodies which are not precipitable, agglutinable, lytic or wheal-producing may be formed in high titer against many antigens.[1,68,104,105]

Allergic subjects who form reagins may also form inhibiting or blocking antibody.[106] Like most skin-sensitizing antibodies, it is non-precipitating. Certain kinds of blocking antibody may be coprecipitated when added to specific precipitates.[107] In contrast to reagin, blocking antibody disappears rapidly from injected skin sites. Some subjects immunized with diphtheria toxoid form a non-precipitating antitoxin which behaves as blocking antibody.[107] It does not remain at skin sites for more than two days as demonstrated by failure to neutralize Schick toxin intradermally. Blocking antibody also differs from reagin in retaining activity after prolonged heating at 56°c.; thus the designation thermostable neutralizing antibody[108] has been applied to it. These differences between antibodies in regard to heat lability have been used to eliminate heat labile reagin from sera of hay fever patients and permit the separate demonstration of blocking antibody.[108] However, these experiments cannot rule out the possibility that heated reagin retains its capacity to combine with allergen but loses its wheal-producing property. Indeed, it has been shown by Kuhns and Pappenheimer[71] that heating at 56°c. quantitatively converts skin-sensitizing antitoxin to an antitoxin which blocks immediate wheal reactions. When mixed with equal amounts of its antigen, blocking antibody inhibits immediate wheal and erythema reactions at sensitized skin sites. One may, in fact, titer sera on the basis of this characteristic.

Blocking antibody may or may not be produced following injection of large amounts of pollen and other extracts into patients.[109] Loveless[110] showed that high titers of blocking antibody were formed in three normal subjects who received repeated injections of ragweed pollen extracts. It has been suggested that the beneficial clinical effects of so-called desensitization treatment for allergy results from the formation of blocking antibodies.

Antibodies which combine with particulate antigens in the presence of physiologic saline solution are sometimes referred to as complete or multivalent antibodies. Immune bodies which do not cause agglutination of their antigens in physiologic saline solution have been described by various terms such as agglutinoids, "low grade," inhibiting, "blocking" or "incomplete" antibodies.[105,111–113] The term univalent has also been applied, possibly incorrectly. Anti-Rh "blocking" antibodies when combined with Rh positive red cells in the presence of saline solution do not agglutinate these cells, nor can agglutination be made to occur if complete Rh antibodies are added to the mixture. This provides the basis for a hemagglutination inhibition test described by Wiener.[112] Direct agglutination of red cells with specifically attached blocking antibodies can be achieved if the cells are suspended in a medium with high protein concentration, that is, 20 to 30 per cent human or bovine albumin, serum, plasma or mixtures of serum and albumin.[114–116] The use

of high molecular weight non-protein materials such as acacia, pectin, polyvinylpyrrolidine, dextran, and the like to detect blocking antibodies has been described.[117–119] Enzyme-modified red blood cells suspended in saline solution[120,121] also may be agglutinated by blocking antibodies. These technics are useful in the detection of blood group incompatibilities occurring in the course of transfusion therapy or in pregnancies complicated by maternal-fetal differences in blood factors.[122–129] Blocking antibody technics have also been extended to certain bacterial systems.[130]

Qualitative differences between "incomplete" antibodies have been recognized.[123,131] Some atypical hemagglutinins, that is, "cryptagglinoids,"[132,133] are detected only by means of anti-antibodies (Coombs technic) or enzyme technics and not by the use of red cells suspended in protein-containing solutions. Some[35] believe that differences between titers of incomplete antibodies as determined by the various technics are not necessarily indications of different antibodies, but may merely reflect the sensitivity of the test used. It is difficult to provide a satisfactory answer to this problem until individual purified blood factor substances are available. Indeed evidence exists that the complex antigenic structure of the red cell envelope may influence the reactivity of atypical hemagglutinins. Race, Sanger and Selwyn[134,135] have compared the serologic reactions shown by $\frac{-D-}{-D-}$ red cells (cells with chromosome deletions for factors C, c, E, e and d) and Rh positive red cells which did not contain chromosome deletions. They demonstrated that incomplete anti-D antibodies agglutinated $\frac{-D-}{-D-}$ cells suspended in saline solution but did not agglutinate the control red cells under the same conditions. However, further studies by Sturgeon[136,137] suggested that agglutination of $\frac{-D-}{-D-}$ cells in saline solution by incomplete anti-D sera may depend upon the presence in the incomplete antiserum of relatively small amounts of complete (multivalent) antibodies. The normal red cell used for purposes of comparison was of the antigenic composition $\frac{cDe}{cde}$.

Anti-antibodies: Antibodies possess a twofold specificity. On the one hand they are species specific, and on the other they are immunologically specific for a definite antigen. Thus rabbit anti-ovalbumin is a typical rabbit gamma globulin, precipitable by antirabbit globulin sera. It has been possible to make use of this dual specificity for many purposes but only a few examples can be mentioned here. In order to determine the relative gamma globulin content of three horse serum globulin fractions which contained tetanus antitoxin, Jager, Smith, Bernhisel and Jager[138] prepared rabbit antisera against them. Precipitation technics were employed using rabbit anti-horse globulin as antibody and the different antitoxin-containing globulin components as antigens. The gamma and T-globulin fractions were shown to be closely related immunologically when the quantitative precipitin technic was employed. The third fraction, which contained predominantly beta globulin, behaved differently as compared with gamma and T-globulins.

Anti-antibodies have been of considerable interest in the study of abnormal proteins formed in multiple myeloma. For example, rabbit antisera produced against normal human gamma globulin may precipitate myeloma proteins.[139] However, absorption of gamma globulin antiserum with purified myeloma proteins removes only part of the total antibody. Occasionally, antisera against a myeloma protein possess a highly individual character and may not react with normal gamma globulins or with other myeloma proteins.[3] Rabbit anti-gamma globulin does not form a precipitate or does so only slightly when mixed with sera from persons who possess little or no gamma globulin.[179] This defect may occur in agammaglobulinemia and occasionally in other diseases associated with serum protein abnormalities.

Incomplete antibodies may be detected in immune sera by the use of an antihuman globulin technic. A soluble mixture of human non-precipitating antibody and its antigen may be precipitated by the addition of rabbit anti-human gamma globulin. Particulate antigens in the presence of blocking antibodies may be agglutinated following addition of the same reagent. Coombs, Mourant and Race[140] showed that this test was of importance in detecting Rh antibodies of the incomplete type. Anti-Rh serum was added to Rh positive cells; the cells were then washed free of proteins other than specifically attached antibody, and rabbit antihuman globulin was then added. This was

followed by rapid agglutination of the red cells. The red cells of infants with hemolytic disease caused by Rh incompatibility or other blood factor differences are already combined with antibodies. Agglutination of the red cells of these infants can be made to occur simply by washing the cells and exposing them to anti-globulin serum.

4. *Abnormal Substances Participating in Immune Phenomena.* During the course of most infections, inflammatory conditions and occasionally in other diseases, blood components termed acute phase materials may be found. They include certain high molecular weight carbohydrates such as mucoproteins and glycoproteins,[141–143] amino sugars[144–146] and carbohydrate-protein complexes.[147–149] Electrophoretic analysis of acute phase serum or plasma may show changes in alpha and beta globulin fractions[150,151,152] or in fibrinogen.[153] A non-specific inhibitor of hyaluronidase has also been described.[154]

Another important acute phase substance is C-reactive protein.[155] This protein, which occurs in serum intimately linked to lipid, is precipitable in the presence of calcium ions when added to somatic C-polysaccharide from pneumococci.[156–159] It may also be precipitated by an anti-C protein which can be prepared by immunization of rabbits. Since C-reactive protein is an abnormally occurring component, the tests for its presence provide the basis for an important diagnostic procedure in acute inflammatory states such as rheumatic fever.[160]

Wood[161] carried out studies in rabbits immunized with human C-protein or human gamma globulin. He found a correlation between the amount of rabbit C-reactive protein produced and the subsequent production of significant titers of antibody.

THE VALENCE OF ANTIBODIES

The precipitin reaction occurs in two stages which are in practice inseparable from one another: (1) primary specific combination between antigen and antibody, and (2) specific aggregation of the primary complex.[162–164] Since antigens generally contain multiple specific combining sites, the sequence of events leading to aggregation can take place because the antibody molecules possess more than one combining group for antigens.[165–168] The number of immunologically reactive sites on an antibody is referred to as valence. Detailed analyses of antigen-antibody complexes in antigen excess indicate that antibody has a valence of two.[166,169]

Although many antibodies of biologic interest cause visible reactions such as precipitation, agglutination or lysis, many do not. The question arises as to whether the latter possess only one combining site (univalent) which could account for their lack of visible reactions. The fact that non-specific factors (proteins, high molecular weight polysaccharides) may influence the agglutinating powers of some anti-Rh blocking sera makes it unlikely that these antibodies are really univalent. In other instances in which presumed univalent antibody occurs it is difficult to preclude the possibility that non-specific serum factors hinder aggregation. The work of Kleczkowski,[170,171] and of Cohn and Pappenheimer[60] with heated antisera, of Horsfall and Goodner,[172] of Katsura,[173] and of Kruger and Heidelberger[174] using lipid extracted sera suggest that suitable treatment of some non-precipitating antibodies may result in precipitating antibody. It is of interest that lipid extraction[175] of sera containing non-precipitating skin sensitizing diphtheria antitoxin did not result in appreciable alteration of its characteristics.[176] Evidence can be found which favors univalence of skin sensitizing antitoxin. It fixes complement poorly or not at all and also does not cause a Danysz reaction when antigen is added in increments to a given quantity of antibody.[71] The same results are obtainable when either whole serum or highly purified gamma globulin is used for these experiments.[176] On the other hand, it has been shown by Kabat[64] and Maurer[96] for the dextran system, and by Vaughan and Kabat[36] for the egg white system, that skin reactivity against these antigens (ability to cause immediate wheal reactions) is associated with multivalent antibodies. The problem will lend itself to additional experimental approaches when highly purified non-precipitating and precipitating antibody preparations are made available for comparative biologic studies.

DISTRIBUTION OF ANTIBODIES

Most human antibodies seem to be contained within the gamma globulins. Clinical evidence in favor of this has been provided by studies in patients with agammaglobulinemia,[177–179] a disease which in its extreme form is characterized by complete absence of serum gamma

globulin and of demonstrable antibodies. Much of the work described in this section will be concerned with the partitioning of antibodies within the gamma globulin fraction by chemical, physical or biologic methods.

1. Physical and Chemical Methods of Separation. Comparative studies carried out on the chemical nature of antibodies have agreed generally that they do not differ in chemical properties from normal gamma globulin. For example, Smith and Greene[180] and Smith, McFadden, Stockell and Buettner-Janusch[181] found that rabbit antibodies contained the same amino acids as non-immune gamma globulins. Although the specific arrangement of all acids in an antibody has not yet been determined, Porter[182] and McFadden and Smith[183] found that the terminal arrangement of five amino acids in rabbit gamma globulin containing anti-ovalbumin was identical with that of immunologically inactive rabbit gamma globulin. The molecular weight of most antibodies in human immune serum is the same as that of normal gamma globulin, close to 160,000. Antibodies resemble other proteins in their susceptibility to a large number of denaturing and destructive materials.[163]

Pauling[164] believes that the modifications which characterize antibodies from non-immune globulin derive from a different configuration in space of the polypeptide chain without any alteration in the chain of amino acid residues. On the other hand, Alexander,[184] Mudd[185] and Breinl and Haurowitz[186] have postulated that amino acids at the active portion of an antibody molecule became rearranged. Certain physical and chemical variations among antibodies make it possible to separate them by several procedures. These include differences in isoelectric point, varying resistance to proteolytic enzymes, heat, and the like, and occasionally differences in molecular weight or size or shape, as will be described in the next paragraphs. Sometimes these or other differences may be greatly altered following combinations of antigen with antibody, and the complex can then be separated by chemical or physical procedures.

Technics of separation have made use of the fact that separation of individual proteins in mixtures can be accomplished if the isoelectric point of each component is not the same as that of its neighbor. The serum proteins are essentially colloidal solutions and owe part of their stability to the similar charges carried by their particles. The degree of charge may differ from protein to protein. When these charges are neutralized by ions of opposite charge, one or more proteins may precipitate from solution.

Removal of salts by dialysis or the addition of salts, may cause selective precipitation of serum fractions. When dialyzed against water, globulin is separated into a water soluble fraction (euglobulin) and a salt-soluble fraction (pseudoglobulin). Salting out refers to the precipitation of proteins following the addition of ammonium or sodium sulfate or other salts such as sodium chloride, sodium or potassium phosphate, and compounds containing zinc and barium. The globulin referred to as euglobulin in this procedure precipitates when serum becomes 33 per cent saturated with ammonium sulfate. Pseudoglobulins are precipitated between 33 to 50 per cent saturation with ammonium sulfate. Both fractions represent globulin mixtures and each fraction contains a certain amount of gamma globulin. Antitoxins formed in horses following subcutaneous injection of toxoids are contained in the pseudoglobulin fraction; antibacterial antibodies produced as a consequence of intravenous injection of bacteria, bacterial nucleoproteins or polysaccharides are largely contained in the euglobulin fraction.[40,41,187] Human antibodies are present in the pseudoglobulin and euglobulin fractions.[188–191] Experiments of Stull[192] and Sherrer[189] showed that the skin-sensitizing activity of the sera of allergic patients was located predominantly in the pseudoglobulin fraction. Albumin fractions obtained by dialysis or salt fractionation are usually free of antibody activity.[189–192]

The precise separation of many serum components may be accomplished by the *use of cold alcohol*,[193–196] a technic providing for stepwise removal of different serum or plasma fractions under controlled conditions of pH, ionic strength, temperature, protein concentration and alcohol concentration. Most antibodies are found in fraction II which is almost 100 per cent electrophoretically pure gamma globulin.[197] However, isohemagglutinins, typhoid anti-O antibodies and syphilitic reagin are concentrated in fraction III-1 which is predominantly fast-moving gamma globulin.[197,198] Skin-sensitizing antibodies seem to be associated with fractions which possess gamma globulin[199,200] but some methods used to partition the fractions may harm the wheal and erythema producing property. This is suggested by the

variability in yields of skin-sensitizing antibody in fractions derived by different alcohol precipitation technics.[199–201] Fractionations carried out on sera containing skin-sensitizing diphtheria antitoxin may have a bearing on these results.[200] Fraction II and III (electrophoretically 80 per cent gamma globulin) separated by method 5 was recovered at pH 6.6 and ionic strength close to 0.1. Seventy-five per cent of the original skin-sensitizing antitoxin was recovered as demonstrated by passive transfer studies, and all of it was contained within this fraction. If fraction II was removed by another cold alcohol procedure[196] which exposed the serum proteins in the course of fractionation to pH values as low as 4.7 and 0.01 ionic strength, the total recoverable skin activity was greatly diminished. Further experiments designed to test the role of pH and ionic strength indicated that they were of importance in the preservation of wheal and erythema producing activity of skin sensitizing antitoxin. It was found that sera exposed for various periods to buffers at pH 4.5 or ionic strength 0.01 lost much of their wheal and erythema producing activity. The precipitability of precipitating antitoxic whole sera or gamma globulin was also lost under certain conditions of pH and ionic strength. However, the ability to neutralize toxin was not appreciably impaired, despite the loss of other characteristics of these antibodies.[202]

Electrophoresis is probably the most convenient reference procedure used in study of the antibody-containing globulins and is often used in conjunction with chemical fractionation. It is based upon the finding[203] that proteins in solution migrate at different rates at a selected pH under the influence of an electric current. Electrophoresis of serum is generally carried out over a designated period of time using barbital buffer at pH 8.6 and ionic strength 0.1.[204] Under these conditions serum is separated into albumin, $alpha_1$, $alpha_2$, beta and gamma globulins.

When some animals receive a course of immunization the response may be observed electrophoretically by a rise either in total gamma globulin or in T-globulin, or in both substances. T-globulin is a component intermediate in mobility between beta and gamma globulins.[42,205–207] T-globulins are also designated as $gamma_1$ globulins or fast-moving gamma globulins. Different kinds of antitoxins in animals and humans are present in both $gamma_2$ and $gamma_1$ or T-globulins.[208–210] Most antibody formed by the horse against Hemophilus pertussis is in the $gamma_2$ component.[210] Horse antibodies specifically precipitated by pneumococcal polysaccharide migrate as T or $gamma_1$ globulins.[205]

Under conditions of immunization of human subjects one infrequently observes a titer of sufficient magnitude to result in a protein peak definable upon electrophoresis. Indeed, antibodies which are immunologically identifiable ordinarily constitute an infinitesimal fraction of the gamma globulin, e. g. diphtheria antitoxin, 0.003 gm./L. However, fractions which contain antibody may be concentrated first by chemical separation, and antibody distribution within these concentrates may then be studied by means of electrophoresis.

When the classic Tiselius technic is used for protein analysis the proteins migrate in a fluid medium, a fact which makes it difficult to isolate antibodies from whole serum unless preliminary chemical separations have first been carried out. Zone electrophoresis[211,212] is based upon the same principle but separations are carried out in a solid medium of paper, starch and other material.[213–215] This has simplified the procedure of studying the electrophoretic distribution of antibodies. Sera containing antibodies are placed in a suitably prepared starch or cellulose mold moistened with buffer or an electric current is applied. When proteins have migrated the desired distance the separated globulins containing antibody can be eluted from the supporting material and tested for antibody. Antibodies against viruses,[216] an insulin-neutralizing factor,[217] naturally occurring isohemagglutinins and atypical hemagglutinins[218] have been studied by this method. It is of interest that skin-sensitizing diphtheria antitoxin migrates as a $gamma_1$ globulin[209] and non-sensitizing blocking antitoxin behaves as $gamma_2$ globulin.[219] Differences have also been observed with respect to viral antibodies and their inhibitors.[216] The fact that zone electrophoresis provides a direct and gentle means of separation gives it advantages over other methods when one is concerned with the rapid isolation of biologically active materials.

Electrophoresis-convection,[220–222] a physical separatory method, is based upon principles of the thermal diffusion method employed in the separation of isotopes. It is useful as a precise means of partitioning proteins within a given

serum fraction. For example, purified gamma globulin (fraction II) is separable into four fractions of different mean mobilities and isoelectric points.[222] Using this approach Loveless demonstrated that skin-sensitizing antibodies against insulin and pollen are contained in the $gamma_1$ or beta globulin in contrast to blocking antibodies which are localized in the slow-moving $gamma_2$ globulin.[223,224] Anti-Rh blocking antibodies contained in serum separated by electrophoresis-convection are said to reside in slow-moving gamma globulin, and cryptagglutinoids are distributed throughout the gamma globulin and are found occasionally in other globulins.[225]

An interesting technic incorporating two separatory methods has been described by Williams and Grabar.[226–228] It combines zone electrophoresis in a gelified media with immunochemical analysis in gels by a modification of the Ouchterlony technic. This technic enables one to determine known and unknown components in mixtures of antigens and antibodies, with their electrophoretic characteristics. Williams and Grabar used this method to study immune horse sera, and they demonstrated antibodies which ranged widely in their mobilities from gamma globulin to rapidly moving beta globulin.

Partition chromatography has been utilized by Porter[229] to indicate that changes in distribution of antibody within the gamma globulin occur during immunization of rabbits with ovalbumin, type III pneumococci and influenza virus. Antibody distribution was dependent on the history of immunization, being confined to the slower moving fractions after the first series of injections. Later a second antibody peak became apparent, suggesting production of a different kind of antibody. No evidence could be found of differences in combining ratios between antibodies of different fractions but, when used as antigens and tested against hen antirabbit gamma globulin serum by the Oudin technic, differences were apparent.

In this connection an important use of ion exchange resins and metal ions in qualitative studies of antibodies has been described by Isliker[230] who purified Rh antibodies with these materials. Using chromatographic columns he adsorbed antibody against red cells and then eluted the antibody with solutions of sugars or other chemicals. It was of interest that saline antibodies were precipitated completely by 15 mm. zinc glycinate but blocking antibodies were carried down only to a small extent under these conditions. Removal of zinc from antibody was achieved by a cation exchange resin.

Action of enzymes and other chemicals, heat and irradiation: Antibodies vary in their susceptibility to enzymes or other physical or chemical procedures. Diphtheria antitoxin has been treated with pepsin, papain and bromelin[231–234] without appreciably affecting its neutralizing properties. However, the nature of the antitoxin molecule changes,[234] along with its ability to precipitate in the presence of toxin.[232] Antibody activity in treated immune serum formed against pneumococcal polysaccharides has also been found to persist following treatment with enzymes, although definite differences in certain immunologic properties have been reported.[235,236] Pepsin treatment of horse antipneumococcus serum was observed to destroy most of the mouse protective power but some of the precipitating powers remained.[235] Porter[237] found that under appropriate conditions papain treatment of rabbit antiovalbumin resulted in the formation of an immunologically reactive fragment which inhibited precipitation when in the presence of ovalbumin and its corresponding untreated rabbit antibody. Under conditions in which some antibodies are partially altered by heat at 56°C.[60,170,238] or lipid extraction,[172,174,239] precipitability or agglutinability may be lost, but in certain instances restoration of these properties occurs following treatment with lecithin,[172] trypsin[171] or pepsin.[173] The significance of the observed differences between antibodies with respect to resistance to enzymes,[240,241] heat,[242] acid or alkali[1] and high pressures[243] is unknown. Serum factors unrelated to gamma globulin may be of importance in regard to some of these differences. For example, when whole human serum containing precipitating diphtheria antitoxin is heated at 56°C. there is a loss of precipitability; when purified gamma globulin from the same serum is similarly treated, no loss of precipitability occurs.[60] Skin-sensitizing antitoxic sera loses its properties after heating at 56°C. but it is converted as a result to a form of antitoxin which inhibits the corresponding reactions.[71] On the other hand, heated precipitating diphtheria antitoxin does not inhibit precipitating reactions, but instead can be coprecipitated when appropriate amounts of toxin are added to it.[60] Heat at 56°C. does not alter the anaphylacto-

genic properties of either precipitating or skin-sensitizing antitoxin. Other chemical or physical procedures, such as photo-oxidation,[244,245] irradiation,[246,247] or the acetylation of amino groups,[248] modify the behavior of antibodies. Their specific activity may be destroyed by alcohol at room temperature, ketene or excessive amounts of halogens.[1] When only two to five halogen atoms are introduced into each antibody molecule its specificity is not affected; antibodies can therefore be labelled with radioactive iodine for quantitative or qualitative studies.[249–253] Antibodies can also be labelled with radioactive sulfur without destruction of antibody specificity by coupling the antiserum with diazotized amino benzene sulfonic acid containing S^{35}. (Ref. 254.) Their distribution can then be studied by employing indicators which determine isotope activity.

Study of antibodies in the ultracentrifuge: The sedimentation behavior of antibodies may be determined by subjecting them to relatively high speeds in the ultracentrifuge. The molecular weight is determined by combining data obtained by means of the ultracentrifuge with diffusion measurements through a defined medium under defined physical conditions. Antibodies in the rabbit, monkey and man usually possess a sedimentation constant of about 7 and a molecular weight of about 160,000 or slightly higher.[27,255,256] In the horse, cow and pig corresponding antibodies of about 900,000 molecular weight have been reported.[27,256] However, there are differences between the weights of some antibodies within the same species. Horse diphtheria antitoxin has a sedimentation constant of 7 and a molecular weight of 184,000[165] but horse antipneumococcal antibody has a sedimentation constant of 19 with a molecular weight of over 900,000.[256] In the human, Wassermann antibody contains two components with sedimentation constants of 7 and 19. (Ref. 257.) The molecular weight and sedimentation constants of human isohemagglutinins have been shown to be higher than most antibodies.[258,259] No evidence as yet exists to suggest that sedimentation values for skin-sensitizing antibodies are any different from values obtained for normal human gamma globulin.

2. Antigen-Antibody Complexes in the Study of Antibodies and Their Distribution. Although initial combination between antigens and antibodies seems to occur in a matter of seconds[260,261] subsequent aggregation may take place over a variable period depending upon a number of physical and chemical conditions, or aggregation may be suppressed or may not occur at all, for reasons already described. The firmness of union between antigen and antibody, termed avidity, is frequently a matter of practical importance in the evaluation of diagnostic antisera.[262]

For purposes of learning more about the nature and distribution of antibodies as part of a complex, it is desirable at times to carry out studies on such combinations. The procedure of forming a complex, then dissociating the complex, is at present the simplest method of obtaining practically pure antibody freed of other serum components, since chemical methods are much less precise. Therefore, if an antibody can first be purified by precipitation with its antigen and can then be liberated from its union with antigen, the characteristics of pure antibody may be compared with those of antibody contained in whole serum. The chief disadvantage is that the procedures required to disrupt such a union, that is, high salt concentrations, relatively high degrees of acidity or alkalinity, heat and enzymes, are drastic and may cause a certain amount of denaturation. A number of technics have been described and may be consulted in the original for additional details.[263–268]

Many antibodies of biologic importance which should be studied in the pure state cannot be precipitated or agglutinated by their antigens. Combination between these materials cannot be observed and therefore cannot be manipulated in the test tube. Isotope-labelled antigens may be of use in determining the physical or chemical behavior of combinations which contain non-precipitating antibodies. Comparisons between the electrophoretic characteristics of isotope labelled complexes containing (1) skin-sensitizing diphtheria antitoxin and excess toxin and (2) precipitating antitoxin and excess toxin indicate differences in migration.[269] This study suggests that mixtures of antitoxins combined with toxin may be separated into precipitating and non-precipitating antitoxin complexes. The isolated complexes may then be subjected to comparative analysis by means of other technics. Pappenheimer[270] has described a method for recovering very high molecular weight antibody freed of other proteins after high speed ultracentrifugation. It is conceivable that a combination of antigen and non-precipitating

antibody would be sufficiently heavy to sediment relatively fast at high speeds in the ultracentrifuge. The presence of an isotope label in these preparations would enable one to observe their behavior accurately.

3. Passage of Antibodies Through the Placenta. The placenta is of importance in the distribution of antibodies between maternal and fetal circulations. The structure of the placenta determines how much antibody can cross its boundary.[271] Thus in domestic ungulates passage of antibodies across the placenta does not occur and transfer of passive immunity from mother to young is by way of the colostrum. Antidodies are transferred by way of the uterus in rabbits, guinea pigs and man. Dogs, rats and mice seem to occupy an intermediate position in this respect. Observations made in human subjects show that both qualitative and quantitative differences may exist in regard to the antibody content of maternal and cord sera. Agglutinins for streptococcus MG[272] and the H and O antigens of Escherichia coli[273] have been shown to be higher in maternal blood than in corresponding cord blood. However, the antibody titer of cord or infant blood at birth may equal or exceed that of the mother's blood as shown by studies of staphylococcal antibodies[274] and of diphtheria antitoxin.[275] Studies of blood group agglutinins in maternal cord blood combinations has generally shown that anti-A and anti-B can cross the placenta, but not freely.[276] Longsworth, Curtis and Pembroke[277] demonstrated by means of electrophoresis that the concentration of gamma globulins of fetal sera is generally higher than that of gamma globulins contained in the corresponding maternal sera.

Qualitative differences in antibody transmission across the placenta are known to occur. Skin-sensitizing antibodies formed against different allergic antigens do not cross the placenta[278,279,280] but allergic blocking antibodies can do so.[280] Similar studies have also been carried out on the distribution of antibodies formed against the Rh and other blood factors. Thus it has been shown that the mother may form agglutinating or blocking antibodies, but the cord serum of the infant rarely, if ever, possesses anything but blocking antibodies.[276,281,282] Timmerman[283] demonstrated that antityphoid H agglutinins passed the placenta in a number of cases but O agglutinins were scarcely transmitted at all. It is of interest that some antibodies which are poorly transmitted through placental tissue are contained in gamma$_1$ or fast-moving gamma globulin fractions, whereas antibodies which are readily transmitted are among those contained in gamma$_2$ globulin.[223,224,276,280,283]

Enzyme digestion of diphtheria antitoxin seems to affect its transmissibility through the placenta. Hartley[284] demonstrated that antitoxin in pregnant guinea pigs passed the placenta readily but after peptic digestion failed to do so. Although untreated horse antitoxin passed the guinea pig placenta in only small amounts, enzyme-digested antitoxin did not do so at all. When pregnant women with diphtheria were treated with digested horse antitoxin, no antitoxin was observed in the cord blood of the infant. The passage of antitoxin did not appear to be affected by treatment with ammonium sulfate.

Brambell[271] has made a number of important observations on the placental transmission of proteins and antibodies, using the rabbit as an experimental animal. The method of approach was to employ antibodies as markers of the passage of maternal globulins to the fetus. The results indicated that whole maternal serum globulins, including antibodies, are supplied to the fetal rabbit in the uterus at a rapid rate and that they are adsorbed into the fetal system without preliminary degradation. Foreign proteins, that is heterologous antibodies, were freely adsorbed from maternal serum in the early stages of development but a selectivity with respect to heterologous as compared with homologous antibody occurred between the twentieth and twenty-fourth days of fetal development. The fetal embryonic membranes then became capable of distinguishing between proteins of different specific origin. Observations that the placenta behaves as a selective screening or absorbing agent may be profitably applied toward clarification of basic mechanisms underlying the reactions between cells and circulating antibodies.

Acknowledgments: The author wishes to thank Drs. Frank Dixon and Paul Maurer of the University of Pittsburgh School of Medicine for their helpful criticisms and corrections, and Mrs. Barbara Davidson and Miss Patricia Hyland for their assistance in the preparation of this manuscript.

REFERENCES

1. Boyd, W. C. The Proteins, vol. 1, p. 755. New York, 1954. Academic Press.

2. Haurowitz, F. The mechanisms of the immune response. *Biol. Rev.*, 27: 247, 1952.
3. Smith, E. and Jager, V. The characterization of antibodies. *Ann. Rev. Microbiol.*, 6: 207, 1952.
4. Grabar, P. Immunochemistry. *Ann. Rev. Biochem.*, 19: 453, 1950.
5. Mayer, M. M. Immunochemistry. *Ann. Rev. Biochem.*, 20: 415, 1951.
6. Campbell, D. H. The nature of antibodies. *Ann. Rev. Microbiol.*, 2: 269, 1948.
7. Treffers, H. P. Some contributions of immunology to the study of proteins. *Advances in Protein Chem.*, 1: 69, 1944.
8. Treffers, H. P. Medical Physics, vol. 1. Chicago, 1944. Year Book Publishers.
9. Kabat, E. A. Immunochemistry of the proteins. *J. Immunol.*, 47: 513, 1943.
10. Heidelberger, M. and Kendall, F. E. A quantitative study of the precipitin reaction between type III pneumococcus polysaccharide and purified homologous antibody. *J. Exper. Med.*, 50: 809, 1929.
11. Heidelberger, M. and Kabat, E. A. Chemical studies on bacterial agglutination II. The identity of precipitin and agglutinin. *J. Exper. Med.*, 63: 737, 1936.
12. Wu, H., Cheng, L. H. and Li, C. P. Composition of antigen-precipitin precipitate *Proc. Soc. Exper. Biol. & Med.*, 25: 853, 1928.
13. (*a*) Oudin, J. L'analyse immunochimique qualitative; méthode par diffusion des antigènes au sein de l'immunsérum précipitant gélosé. *Ann. Inst. Pasteur*, 75: 33, 1948; (*b*) Oudin, J. *Ann. Inst. Pasteur*, 75: 109, 1948.
14. Ouchterlony, O. *Arkiv Kemi., Mineral, Geol.*, 26: 1, 1948.
15. Elek, S. D. Serological analysis of mixed flocculating systems by means of diffusion gradients. *Brit. J. Exper. Path.*, 30: 484, 1949.
16. Krusius, F. F. *Arch. für Augenheilkunde.*, 67: 6, 1910.
17. Hektoen, L. and Schulhof, K. Lens precipitins. Antigenic properties of alpha and beta crystallins. *J. Infect. Dis.*, 34: 433, 1924.
18. Metalnikoff, S. *Ann. Inst. Pasteur.*, 14: 577, 1900.
19. Henle, W. Specificity of some mammalian spermatozoa. *J. Immunol.*, 34: 325, 1938.
20. Lewis, J. H. Iso-antigenic properties of alcoholic extracts of brain. *J. Immunol.*, 41: 397, 1941.
21. Schwentker, F. F. and Rivers, T. M. Antibody response of rabbits to injections of emulsions and extracts of homologous brain. *J. Exper. Med.*, 60: 559, 1934.
22. Landsteiner, K. The Specificity of Serological Reactions. Cambridge, 1945. Harvard Press.
23. Landsteiner, K. and Lampl, H. Über die Abhängigkeit der serologischen Spezifizität von der chemischen Struktur. (Darstellung von Antigenen mit bekannter chemischer Konstitution der spezifischen Gruppen.) XII. Über Antigene. *Biochem. Ztschr.*, 86: 343, 1918.
24. Landsteiner, K. and Vander Scheer, J. On influence of acid groups on serological specificity of azoproteins. *J. Exper. Med.*, 45: 1045, 1927.
25. Hooker, S. B. and Boyd, W. C. The existence of antigenic determinants of diverse specificity in a single protein III. Further notes on crystalline hen and duck ovalbumins. *J. Immunol.*, 30: 41, 1936.
26. Heidelberger, M. Kabat, E. A. and Shrivastava, D. L. A quantitative study of the cross reaction of types III and VIII penumococci in horse and rabbit antisera. *J. Exper. Med.*, 65: 487, 1937.
27. Kabat, E. A. and Mayer, M. M. Experimental Immunochemistry. Springfield, Illinois, 1948. Charles C Thomas.
28. Goebel, W. F., Beeson, P. B. and Hoagland, C. L. Chemoimmunological studies on soluble specific substance of pneumococcus. Capsular polysaccharide of type XIV pneumococcus and its relationship to blood group A specific substance. *J. Biol. Chem.*, 129: 455, 1939.
29. Finland, M. and Curnen, E. G. Agglutinins for human erythrocytes in type XIV anti-pneumococcic horse serums. *Science*, 87: 417, 1938.
30. Weil, A. J. and Sherman, E. Antigenic relationships of pneumococci to erythrocytes and organs of men and animals. *J. Immunol.*, 36: 139, 1939.
31. Ivanovies, G. Z. Die immunbiologische Verwandtschaft zwischen dem Anthraxpolysaccharid der Pneumokokkus-Typus-XIV-Kapselsubstanz und der spezifischen Substanz der menschlichen roten Blutkörper der Gruppe A. *Ztschr. f. Immunitätsf.*, 98: 373, 1940.
32. Stokinger, H. E. and Heidelberger, M. Quantitative theory of precipitin reaction. Reaction between mammalian thyroglobulins and antibodies to homologous and heterologous preparations. *J. Exper. Med.*, 66: 251, 1937.
33. Wilson, G. S. and Miles, A. A. Serological differentiation of smooth strains of brucella group. *Brit. J. Exper. Path.*, 13: 1, 1932.
34. Stanley, W. M. and Knight, C. A. The chemical composition of strains of tobacco mosaic virus. *Cold Spring Harbor Symp. Quant. Biol.*, 9: 255, 1941.
35. Kabat, E. A. The unity and diversity of antibodies. In: Pappenheimer, A. M., Jr. The Nature and Significance of the Antibody Response. New York, 1953. Columbia University Press.
36. Vaughan, J. H. and Kabat, E. A. Studies on the antibodies in rabbit antisera responsible for sensitization of human skin I. *J. Exper. Med.*, 97: 821, 1953.
37. (*a*) Cohn, M. Methods in Medical Research, vol. 5. Chicago, 1952. Yearbook Publishers; (*b*) Freund, J. The response of immunized animals to specific and non specific stimuli. In: Pappenheimer, A. M., Jr. The Nature and Significance of Antibody Response. New York, 1953. Columbia University Press.
38. Loveless, M. Immunological studies of pollinosis. III. Fluctuations in antibody titer of normal individuals subcutaneously and intravenously injected with pollen extract over protracted periods. *J. Immunol.*, 44: 1, 1942.
39. Walzer, M. Atopic allergy: reaginic sensitivity. *Ann. New York Acad. Sc.*, 50: 743, 1949.
40. Treffers, H. P., Heidelberger, M. and Freund, J. Antiproteins in horse sera. III. Antibodies to rabbit serum albumin and their reaction with antigen. *J. Exper. Med.*, 86: 83, 1947.

41. Treffers, H. P., Heidelberger, M. and Freund, J. Antiproteins in horse sera. IV. Antibodies to rabbit serum globulin and their interaction with antigen. *J. Exper. Med.*, 86: 95, 1947.
42. Kekwick, R. A. and Record, B. R. Some physical properties of diphtheria antitoxic horse sera. *Brit. J. Exper. Path.*, 22: 29, 1941.
43. van der Scheer, J., Wyckoff, R. W. G. and Clarke, F. H. Electrophoretic analysis of tetanal antitoxic horse sera. *J. Immunol.*, 40: 173, 1941.
44. Pope, C. G., Stevens, M. F., Caspary, E. A. and Fenton, E. L. Some new observations on diphtheria toxin and antitoxin. *Brit. J. Exper. Path.*, 32: 246, 1951.
45. Wiener, A. S. Origin of naturally occurring hemagglutinins and hemolysins. Review. *J. Immunol.*, 66: 287, 1951.
46. Filitti-Wurmser, S., Jacquot-Armand, Y. and Wurmser, R. Sur la combinaison des isohémagglutinines avec les groupes agglutinogènes. *J. de chim. phys.*, 47: 419, 1950.
47. Boyd, W. C. Hemagglutinating substances for human cells in various Egyptian plants. *J. Immunol.*, 65: 281, 1950.
48. Boyd, W. C. and Reguera, R. M. Hemagglutinating substances for human cells in various plants. *J. Immunol.*, 62: 333, 1949.
49. (*a*) Buchner, H. Über die bakterientodtende Wirkung des zellenfreien Blutserums. *Centralbl. f. Bakt.*, 5: 817, 1889; (*b*) Über die nähere Natur der bakterientodtenden Substanz in Blutserum. *Centralbl. f. Bakt.*, 6: 561, 1889.
50. (*a*) Von Fodor, J. Neuere Versuche mit Injection von Bakterien in die Venen. *Deutsches med. Wchnschr.*, 12: 617, 1886; (*b*) Die Fähigkeit des Blutes Bakterien zu vernichten. *Deutsches med. Wchnschr.*, 13: 745, 1887.
51. Nuttall, G. Experimente über die bacterienfeindlichen Einflüsse den thierischen Körpers. *Ztschr. hyg.*, 4: 353, 1888.
52. Nuttall, G. Blood Immunity and Blood Relationship. New York, 1904. Cambridge University Press.
53. Pillemer, L., Blum, L., Lepow, I., Ross, A., Todd, E. and Wardlaw, A. The properdin system and immunity. I. Demonstration and isolation of a new serum protein and its role in immune phenomena. *Science*, 120: 279, 1954.
54. Pillemer, L., Schoenberg, M., Blum, L. and Wurz, L. The properdin system and immunity II. Interaction of the properdin system with polysaccharides. *Science*, 122: 545, 1955.
55. Gitlin, D. The use of ultraviolet absorption spectroscopy in the quantitative precipitin reaction. *J. Immunol.*, 62: 437, 1949.
56. Heidelberger, M. and MacPherson, C. F. C. Quantitative micro-estimation of antibodies in sera of man and other animals. *Science*, 97: 405, 1943.
57. Markham, R. A steam distillation apparatus suitable for micro-kjeldahl analysis. *Biochem. J.*, 36: 790, 1942.
58. Scholander, P. F. Microburette. *Science*, 95: 177, 1942.
59. Pappenheimer, A. M., Jr. Studies on diphtheria toxin and its reaction with antitoxin. *J. Bact.*, 43: 273, 1942.
60. Cohn, M. and Pappenheimer, A. M., Jr. A quantitative study of the diphtheria toxin-antitoxin reaction in the sera of various species including man. *J. Immunol.*, 63: 291, 1949.
61. Heidelberger, M. and Kendall, F. E. Quantitative theory of precipitin reaction. Reaction of pneumococcus specific polysaccharides with homologous rabbit antisera. *J. Exper. Med.*, 65: 647, 1937.
62. Heidelberger, M. and Kendall, F. E. Precipitin reaction between type III pneumococcus polysaccharide and homologous antibody. Quantitative study and theory of reaction mechanism. *J. Exper. Med.*, 61: 563, 1935.
63. (*a*) Heidelberger, M. and Kendall, F. E. Quantitative theory of precipitin reaction. Reaction between crystalline egg albumin and its homologous antibody. *J. Exper. Med.*, 62: 697, 1935; (*b*) Wetter, L. R. and Deutsch, H. F. Immunological studies on egg white proteins. Quantitative immunochemical studies of ovomucoid. *Arch. Biochem.*, 28: 399, 1950.
64. Kabat, E. A. and Berg, D. Dextran. An antigen in man. *J. Immunol.*, 70: 514, 1953.
65. Pappenheimer, A. M., Jr. and Robinson, E. S. Quantitative study of Ramon diphtheria flocculation reaction. *J. Immunol.*, 32: 291, 1937.
66. Hottle, G. A. and Pappenheimer, A. M., Jr. Quantitative study of scarlet fever toxin-antitoxin flocculation reaction. *J. Exper. Med.*, 74: 545, 1941.
67. Hooker, S. B. and Boyd, W. C. Equine antihemocyanin. *Ann. New York Acad. Sci.*, 43: 107, 1942.
68. Pappenheimer, A. M., Jr. Anti-egg albumin antibody in horse. *J. Exper. Med.*, 71: 263, 1940.
69. Pope, C. G. and Stevens, M. F. The preparation of non-flocculating diphtheria antitoxin by absorption of non-antitoxic antibodies. *Brit. J. Exper. Path.*, 34: 241, 1953.
70. Danysz, J. *Ann. Inst. Pasteur.*, 16: 331, 1902.
71. Kuhns, W. J. and Pappenheimer, A. M., Jr. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. II. A comparison between the immunological properties of precipitating and non-precipitating (skin sensitizing) antitoxins. *J. Exper. Med.*, 95: 375, 1952.
72. Stavitsky, A. B. Micro methods for the study of proteins and antibodies. II. Specific applications of hemagglutination and hemagglutination–inhibition reactions with tannic acid and protein treated red blood cells. *J. Immunol.*, 72: 368, 1954.
73. Feinberg, R. J. and Flick, J. A. Simple *in vitro* method for measuring antibodies in serum of hayfever patients. *Federation Proc.*, 13: 492, 1954.
74. Moskowitz, M. and Treffers, H. P. Agglutinin in normal sera for periodate treated red cells. *Science*, 3: 717, 1950.
75. Kipnis, D. M. and Sacks, M. S. Observations on an agglutinin in human serum for perjodate treated erythrocytes. *J. Lab. & Clin. Med.*, 45: 632, 1955.
76. Middlebrook, G. and Dubos, R. J. Specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli. *J. Exper. Med.*, 88: 521, 1948.

77. Waaler, E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. *Acta path. et microbiol. Scandinav.*, 17: 172, 1940.
78. Davidsohn, I. Test for infectious mononucleosis. *Am. J. Clin. Path.*, 2: 56, 1938.
79. Cutbush, M., Falconer, H. and Mollison, P. Cited by Mollison, P. In: Blood Transfusion in Clinical Medicine. Oxford, 1951. Blackwell Scientific Publications.
80. Dacie, J. V. The Hemolytic Anemias. New York, 1954. Grune & Stratton.
81. Mackenzie, G. M. Observations on paroxysmal hemoglobinuria. *J. Clin. Investigation*, 7: 27, 1929.
82. Crosby, W. H. Paroxysmal nocturnal hemoglobinuria. A classic description by Paul Strübing in 1882, and a bibliography of the disease. *Blood*, 6: 270, 1951.
83. Boyd, W. C. Fundamentals of Immunology. New York, 1943. Interscience Publishers, Inc.
84. Leunette, E. H. and Perlowogoru, A. The complement fixation test in the diagnosis of yellow fever. *Am. J. Trop. Med.*, 23: 481, 1943.
85. Felton, L. D. and Stahl, H. J. A method for the standardization of antipneumococcic horse sera and concentrates. *Pub. Health Rep.*, 50: 1730, 1935.
86. Heidelberger, M., Sia, R. H. P. and Kendall, F. E. Specific precipitation and mouse protection in type I antipneumococcus sera. *J. Exper. Med.*, 52: 477, 1930.
87. Fraser, D. T. Technique of method for quantitative determination of diphtheria antitoxin by skin test in rabbits. *Tr. Roy. Soc. Canada*, 25: 175, 1931.
88. Van Heyningen, W. E. Bacterial Toxins. Oxford, 1950. Blackwell Scientific Publications.
89. McCarty, M. Streptococcal Infections. New York, 1954. Columbia University Press.
90. Adams, M. H. Reaction between enzyme tyrosinase and its specific antibody. *J. Exper. Med.*, 76: 175, 1942.
91. (*a*) Kirk, J. S. and Sumner, J. B. Reaction between crystalline urease and antiurease. *J. Biol. Chem.*, 94: 21, 1931; (*b*) Antiurease. *J. Immunol.*, 26: 495, 1934.
92. Campbell, D. H. and Fourt, L. Immunochemistry of catalase. *J. Biol. Chem.*, 129: 385, 1939.
93. Prausnitz, C. and Küstner, H. Studien über die Ueberempfindlichkeit. *Centralbl. f. Bakt.*, 86: 160, 1921.
94. Coca, A. F. and Grove, E. F. Studies in hypersensitiveness. Study of atopic reagins. *J. Immunol.*, 10: 445, 1925.
95. Lippard, V. W. and Schmidt, W. M. Human passive transfer antibody. II. Neutralization of antigen. *Am. J. Dis. Child.*, 54: 777, 1937.
96. Maurer, P. Personal communication.
97. Kuhns, W. J. and Pappenheimer, A. M., Jr. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. I. Relationship of skin sensitivity to purified diphtheria toxoid to the presence of circulating, non-precipitating antitoxin. *J. Exper. Med.*, 95: 363, 1952.
98. Lowell, F. C. Skin sensitizing antibody and allergy and resistance to insulin. In: Pappenheimer, A. M., Jr. The Nature snd Significance of the Antibody Response. New York, 1953. Columbia University Press.
99. Kabat, E. A. and Landow, H. A. A quantitative study of passive anaphylaxis in the guinea pig. I. *J. Immunol.*, 44: 69, 1942.
100. Benacerraf, B. and Kabat, E. A. A quantitative study of passive anaphylaxis in the guinea pig. V. Latent period in passive anaphylaxis in its relation to dose of rabbit antiovalbumin. *J. Immunol.*, 62, 517, 1949.
101. Kabat, E. A. and Benacerraf, B. A quantitative study of passive anaphylaxis in the guinea pig. IV. Sensitization with non-precipitable or "univalent" rabbit antiovalbumin. *J. Immunol.*, 62: 97, 1949.
102. Benacerraf, B. and Kabat, E. A. A quantitative study of the Arthus phenomenon induced passively in the guinea pig. *J. Immunol.*, 64: 1, 1950.
103. Kuhns, W. J. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. III. Studies of the passive Arthus reaction in guinea pigs using human precipitating and non-precipitating diphtheria antitoxin. *J. Exper. Med.*, 97: 903, 1953.
104. Eisenberg, P. and Volk, R. Untersuchungen über die Agglutination. *Ztschr. f. Infektionskr.*, 40: 155, 1902.
105. Coca, A. F. and Kelly, M. F. Serological study of bacillus of Pfeiffer. *J. Immunol.*, 6: 87, 1921.
106. Cooke, R. A., Barnard, J. H., Hebald, S. and Stull, A. Serological evidence of immunity with co-existing sensitization in type of human allergy (hay fever). *J. Exper. Med.*, 62: 733, 1935.
107. Kuhns, W. J. Immunological properties of a form of non-precipitating diphtheria antitoxin which does not sensitize human skin. Studies of its relation to the inhibition reaction. *J. Immunol.*, 75: 105, 1955.
108. Loveless, M. Immunological studies of pollinosis. Presence of two antibodies related to same pollen —antigen in serum of treated hay fever patients. *J. Immunol.*, 38: 25, 1940.
109. Cooke, R. A., Loveless, M. and Stull, A. Studies on immunity in type of human allergy (hay fever). Serological response of non-sensitive individuals to pollen injections. *J. Exper. Med.*, 66: 689, 1937.
110. Loveless, M. Immunological studies of pollinosis. III. Fluctuations in antibody titer of normal individuals subcutaneously and intravenously injected with pollen extract over protracted periods. *J. Immunol.*, 44: 1, 1942.
111. Race, R. R. An "incomplete" antibody in human serum. *Nature*, 153: 771, 1944.
112. Wiener, A. S. A new test (blocking test) for Rh sensitization. *Proc. Soc. Exper. Biol. & Med.*, 56: 173, 1944.
113. Diamond, L. K. and Abelson, N. M. The importance of Rh inhibitor substance in antiRh serums. *J. Clin. Investigation*, 24: 122, 1945.
114. Weiner, A. S. Conglutination test for Rh sensitization. *J. Lab. Clin. Med.*, 30: 662, 1945.
115. Diamond, L. K. and Denton, R. L. Rh agglutination in various media with particular reference to

the value of albumin. *J. Lab. Clin. Med.*, 30: 821, 1945.

116. Wiener, A. S., Hurst, J. G. and Sonn-Gordon, E. B. Studies on conglutination reaction with special reference to nature of conglutinin. *J. Exper. Med.*, 86: 267, 1947.
117. Levine, P. A. A survey of the significance of the Rh factor. *Blood*, 3: 3, 1948.
118. McNeill, C. and Trentelman, E. F. Detection of Rh antibodies by polyvinylpyrrolidone (PVP). *Am. J. Clin. Path.*, 22: 77, 1952.
119. Grubb, R. Dextran as a medium for the demonstration of incomplete anti Rh agglutinins. *J. Clin. Path.*, 2: 223, 1949.
120. Morton, J. A. and Pickles, M. M. The use of trypsin in the detection of incomplete anti Rh antibodies. *Nature*, 159: 779, 1947.
121. Kuhns, W. J. and Bailey, A. Use of red cells modified by papain for detection of Rh antibodies. *Am. J. Clin. Path.*, 20: 1067, 1950.
122. Levine, P., Burnham, L., Katzin, E. M. and Vogel, P. The role of isoimmunization in the pathogenesis of erythroblastis fetalis. *Am. J. Obst. & Gynec.*, 42: 925, 1941.
123. Levine, P., Vogel, P. and Rosenfield, R. Hemolytic Disease of the Newborn. Advances in Pediatrics, vol. 6. Chicago, 1953. Yearbook Publishers.
124. Potter, E. Rh, Its Relation to Hemolytic Disease. Chicago, 1947. Yearbook Publishers.
125. Pickles, M. M. Hemolytic Disease of the Newborn. Oxford, 1949. Blackwell Scientific Publications.
126. Mollison, P. Blood Transfusion in Clinical Medicine. Oxford, 1951. Blackwell Scientific Publications.
127. Diamond, L. K., Allen, F. H., Jr., Vann, D. D. and Powers, J. R. Round table discussion: erythroblastosis fetalis. *Pediatrics*, 10: 337, 1952.
128. Wiener, A. S. Results of therapy of erythroblastosis with exchange transfusion. *Blood*, 4: 1, 1949.
129. Wiener, A. S. Rh-Hr Blood Types, Applications in Clinical and Legal Medicine and Anthropology. New York, 1954. Grune & Stratton.
130. Griffiths, J. J. Agglutination and agglutinin-"blocking" property in serums from known cases of brucellosis. *Pub. Health Rep.*, 62: 865, 1947.
131. Race, R. R. and Sanger, R. Blood Groups in Man, 2nd ed. Springfield, Illinois, 1954. Charles C Thomas.
132. Hill, J. M. and Haberman, S. Demonstration of Rh antibodies in the newborn and further evidence of the pathogenesis of erythroblastosis fetalis. *J. Lab. & Clin. Med.*, 31: 1053, 1946.
133. Hill, J. M., Haberman, S. and Guy, R. Further evidence for antibodies of third order. *Am. J. Clin. Path.*, 19: 134, 1949.
134. Race, R. R., Sanger, R. and Selwyn, J. G. A probable deletion in a human Rh chromosome. *Nature*, 166: 520, 1950.
135. Race, R. R., Sanger, R. and Selwyn, J. G. Possible deletion in human Rh chromosome. Serological and genetical study. *Brit. J. Exper. Path.*, 32: 124, 1951.
136. Sturgeon, P. Studies of a "complete" and an "incomplete" Rh antiserum with cDe/cDe and —D—/—D— cells, native sera. *J. Immunol.*, 68: 277, 1952.
137. Sturgeon, P. and Brown, R. A. Studies of a "complete" and an "incomplete" Rh antiserum II. Fractions of the "incomplete" serum. *J. Immunol.*, 68: 287, 1952.
138. Jager, B. V., Smith, E. L., Bernhisel, H. and Jager, L. Immunological studies of three antitoxin containing globulin fractions of horse sera. *J. Immunol.*, 65: 311, 1950.
139. Kunkel, H. G., Slater, R. J. and Good, R. A. Relation between certain myeloma proteins and normal gamma globulin. *Proc. Soc. Exper. Biol. & Med.*, 76: 190, 1951.
140. Coombs, R. R. A., Mourant, A. E. and Race, R. R. A new test for the detection of weak and "incomplete" Rh agglutinins. *Brit. J. Exper. Path.*, 26: 255, 1945.
141. Mayer, K. Über eine mucoidähnliche Substanz aus Serum. *Ztschr. f. physiol. Chem.*, 275: 16, 1942.
142. Winzler, R., Devor, A., Mehl, J. and Smythe, I. Studies on mucoproteins of human plasma. Determination and isolation. *J. Clin. Investigation*, 27: 609, 1948.
143. Kelley, V., Good, R. and McQuarrie, I. Serum mucoproteins in children in health and disease with special reference to rheumatic fever. *Pediatrics*, 5: 824, 1950.
144. West, R. and Clarke, D. H. Concentration of glucosamine in normal and pathological sera. *J. Clin. Investigation*, 17: 173, 1938.
145. Rosenberg, C. and Schloss, B. Plasma hexosamine levels in acute rheumatic fever. *Am. Heart J.*, 38: 872, 1949.
146. Kelley, V. Acute phase reactants. Serum hexosamines in patients with rheumatic fever and related diseases. *J. Pediat.*, 40: 413, 1952.
147. Nilsson, I. Über den Glucosamingehalt des Blutserums bei Gesunden und Pneumonierkranken. *Biochem. Ztschr.*, 291: 254, 1937.
148. Blix, G., Tiselius, A. and Svennson, H. Lipids and polysaccharides in electrophoretically separated blood serum proteins. *J. Biol. Chem.*, 137: 485, 1941.
149. Shetlar, M., Foster, J., Kelley, K., Shetlar, C., Bryan, R. and Everett, M. Serum polysaccharide level in malignancy and in other pathological conditions. *Cancer Res.*, 9: 515, 1949.
150. Longsworth, L., Shedlovsky, T. and MacInnes, D. Electrophoretic patterns of normal and pathological human blood serum and plasma. *J. Exper. Med.*, 70: 399, 1939.
151. Blix, G. Quantitative Bestimmung von electrophoretisch getrennten Serumglobulinen. *Ztschr. f. d. ges. exper. Med.*, 105: 595, 1939.
152. Shedlovsky, T. and Scudder, J. Comparison of erythrocyte sedimentation rates and electrophoretic patterns of normal and pathological human blood. *J. Exper. Med.*, 75: 119, 1942.
153. Gram, H. C. *Acta med. Scandinav.*, 50: 107, 1922.
154. Good, R. A. Acute phase substances produced in rheumatic diseases. In: Rheumatic Fever. Minneapolis, 1952. University of Minnesota Press.
155. Tillett, W. S. and Francis, T., Jr. Serological reactions in pneumonia with non-protein somatic

fraction of pneumococcus. *J. Exper. Med.*, 52: 561, 1930.

156. Abernathy, T. J. and Avery, O. T. Occurrence during acute infections of protein not normally present in blood. Distribution of reactive protein in patients' sera and effect of calcium on flocculation reaction with C-polysaccharide of pneumococcus. *J. Exper. Med.*, 73: 173, 1941.
157. MacLeod, C. M. and Avery, O. T. Occurrence during acute infections of protein not normally present in blood. Isolation and properties of reactive protein. *J. Exper. Med.*, 73: 183, 1941.
158. MacLeod, C. M. and Avery, O. T. Occurrence during acute infections of protein not normally present in blood. Immunological properties of C-reactive protein and its differentiation from normal blood proteins. *J. Exper. Med.*, 73: 191, 1941.
159. McCarty, M. The occurrence during acute infections of a protein not normally present in the blood. IV. Crystallization of the C-reactive protein. *J. Exper. Med.*, 85: 491, 1947.
160. Anderson, H. C. and McCarty, M. Determination of C-reactive protein in blood as measure of activity of disease process in acute rheumatic fever. *Am. J. Med.*, 8: 445, 1950.
161. Wood, H. F. The relationship between the acute phase response and antibody production in the rabbit. I. Correlation between the early appearance of C-reactive protein and subsequent antibody production. *J. Exper. Med.*, 98: 311, 1953.
162. (*a*) Heidelberger, M. Chemical aspects of the precipitin and agglutinin reactions. *Chem. Rev.*, 24: 323, 1939; (*b*) Quantitative absolute methods in the study of antigen antibody reactions. *Bact. Rev.*, 3: 49, 1939.
163. Marrack, J. R. Chemistry of Antigens and Antibodies, 2nd ed. Great Britain Medical Research Council. Spec. Rep. Series No. 230 1938.
164. Pauling, L. Theory of structure and process of formation of antibodies. *J. Am. Chem Soc.*, 62: 2643, 1940.
165. Pappenheimer, A. M., Jr., Lundgren, H. P. and Williams, J. W. Studies on molecular weight of diphtheria toxin, antitoxin, and their reaction products. *J. Exper. Med.*, 71: 247, 1940.
166. Marrack, J. R., Hoch, H. and Johns, R. G. S. Valency of antibodies. *Brit. J. Exper. Path.*, 32: 212, 1951.
167. Eisen, H. N. and Karush, F. The interaction of purified antibody with homologous hapten. Antibody valence and binding constant. *J. Am. Chem. Soc.*, 71: 363, 1949.
168. Pauling, L., Pressman, D. and Campbell, D. H. Serological properties of simple substances. Precipitin of mixture of two specific antisera by dihaptenic substance containing two corresponding haptenic groups. Evidence for framework theory of serological precipitin. *J. Am. Chem. Soc.*, 66: 330, 1944.
169. Plescia, O. J., Becker, E. L., Williams, J. W. Valence of precipitating antibodies. *J. Am. Chem. Soc.*, 74: 1362, 1952.
170. Kleczkowski, A. Effect of heat on flocculating antibodies of rabbit antisera. *Brit. J. Exper. Path.*, 22: 192, 1941.
171. Kleczkowski, A. Restoration of the ability of some heated antisera to flocculate specifically their antigens. *Brit. J. Exper. Path.*, 31: 145, 1950.
172. (*a*) Horsfall, F. and Goodner, K. Lipoids and immunological reactions. Relation of phospholipins to type-specific reactions of antipneumococcus horse and rabbit sera. *J. Exper. Med.*, 62: 485, 1935; (*b*) Lipoids and immunological reactions. III. Lipid content of specific precipitates from type I antipneumococcus sera. *J. Exper. Med.*, 64: 583, 1936.
173. Katsura, T. Immunochemical studies on diphtheria antitoxin. II. Effect of the extraction of lipids on the flocculation reaction. *Jap. J. Exper. Med.*, 20: 657, 1950.
174. Kruger, R. C. and Heidelberger, M. Effect of removal of lipids on specific precipitation. *J. Exper. Med.*, 92: 383, 1950.
175. Blix, G. Electrophoresis of lipid free blood serum. *J. Biol. Chem.*, 137: 495, 1941.
176. Kuhns, W. J. Unpublished observations.
177. Bruton, O. C. Agammaglobulinemia. *Pediatrics*, 9: 722, 1952.
178. Good, R. A. Clinical investigations in patients with agammaglobulinemia. *J. Lab. Clin. Med.*, 44: 803, 1954.
179. Janeway, C. and Gitlin, D. Agammaglobulinemia. Diagnosis and management of a new disease entity. *Medical News*, 1: 1, 1955.
180. Smith, E. and Greene, R. Further studies on amino acid composition of immune proteins. *J. Biol. Chem.*, 171: 355, 1947.
181. Smith, E., McFadden, M. L., Stockell, A. and Buettner-Janusch, V. Amino acid composition of four rabbit antibodies. *J. Biol. Chem.*, 214: 197, 1955.
182. Porter, R. R. A chemical study of rabbit antiovalbumin. *Biochem. J.*, 46: 473, 1950.
183. McFadden, M. L. and Smith, E. L. Free amino groups and N-terminal sequence of rabbit antibodies. *J. Biol. Chem.*, 214: 185, 195 .
184. Alexander, J. Some intracellular aspects of life and disease. *Protoplasma.*, 14: 296, 1 32.
185. Mudd, S. A hypothetical mechanism of antibody formation. *J. Immunol.*, 23: 423, 1932.
186. Breinl, F. and Haurowitz, F. Chemische Untersuchung des Präzipitates aus Hämoglobin und Anti-Hämoglobin-Serum und Bemerkungen über die Natur der Antikörper. *Ztschr. f. physiol. Chem.*, 192: 45, 1930.
187. Heidelberger, M. Antiproteins in horse sera. Antibodies to pneumococcus nucleoprotein and their reaction with antigen. *J. Exper. Med.*, 86: 77, 1947.
188. Avery, O. T. Distribution of the immune bodies occurring in the antipneumococcus serum. *J. Exper. Med.*, 21: 133, 1925.
189. Sherrer, E. L. Distribution of reagins in the blood plasma. *J. Allergy*, 2: 467, 1931.
190. Kendrick, P. L. and Kahn, R. Precipitation with fractions of syphilitic serum and arachnoid fluid. *J. Infect. Dis.*, 39: 202, 1926.
191. Witebsky, E., Mohn, J. F., Howles, D. J. and Ward, H. M. Simple method for concentration of Rh agglutinins. *Proc. Soc. Exper. Biol. & Med.*, 61: 1, 1946.

192. Stull, A., Sherman, W. and Cooke, R. A. The association with pseudoglobulin of the skin sensitizing substance of allergic (hay fever) serum. *J. Allergy*, 9: 7, 1937.
193. Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. L., Jr. and Armstrong, S. H., Jr. Chemical, clinical and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. *J. Clin. Investigation*, 23: 417, 1944.
194. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. L., Ashworth, J. N., Melin, M. and Taylor, H. L. Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J. Am. Chem. Soc.*, 68: 459, 1946.
195. Deutsch, H. F., Gasting, L. J., Alberty, R. A. and Williams, J. W. Biophysical studies of blood plasma proteins. Recovery of gamma globulin from human blood protein mixtures. *J. Biol. Chem.*, 164: 109, 1946.
196. Deutsch, H. F., Alberty, R. A. and Gosting, L. J. Biophysical studies of blood plasma proteins. Separation and purification of new globulin from normal human plasma. *J. Biol. Chem.*, 165: 21, 1946.
197. Enders, J. F. Concentrations of certain antibodies in globulin fractions derived from human blood plasma. *J. Clin. Investigation*, 23: 510, 1944.
198. Oncley, J. L., Melin, M., Richert, D. A., Cameron, J. W. and Gross, P. M., Jr. The separation of the antibodies, isoagglutinins, prothrombin, plasminogen and beta lipoproteins into subfractions of human plasma. *J. Am. Chem. Soc.*, 71: 541, 1949.
199. Menzel, A. E. O., Kessler, W. R., Cooke, R. A. and Myers, P. Immuno-serologic studies of the immediate wheat type allergies. *J. Allergy*, 23: 483, 1952.
200. Kuhns, W. J. Immunochemical properties of serum fractions of human non-precipitating diphtheria antitoxin. *Federation Proc.*, 12: 1480, 1953.
201. Vaughan, J. H., Favour, C. B. and Jaffee, I. H. Partition of the skin sensitizing antibody among alcohol-fractionated plasma proteins. *J. Allergy*, 23: 489, 1952.
202. Kuhns, W. J. Unpublished observations.
203. Tiselius, A. Electrophoresis of serum globulin. Electrophoretic analysis of normal and immune serum. *Biochem. J.*, 31: 1464, 1937.
204. Longsworth, L. G. Recent advances in the study of proteins by electrophoresis. *Chem. Rev.*, 30: 323, 1942.
205. Tiselius, A. and Kabat, E. A. Electrophoretic study of immune sera and purified antibody preparations. *J. Exper. Med.*, 69: 119, 1939.
206. van der Scheer, J., Wyckoff, R. W. G. and Clarke, F. G. Electrophoretic analysis of several hyperimmune horse sera. *J. Immunol.*, 39: 65, 1940.
207. Moore, D. H., van der Scheer, J. and Wyckoff, R. W. G. Electrophoretic study of antipneumococcal horse sera. *J. Immunol.*, 38: 221, 1940.
208. Smith, E. L. and Gerlough, T. D. Isolation and properties of proteins associated with tetanus antitoxin activity in equine plasma. *J. Biol. Chem.*, 167: 679, 1947.
209. Kuhns, W. J. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. IV. Differences between human precipitating and non-precipitating skin-sensitizing diphtheria antitoxin as shown by electrophoresis. *J. Exper. Med.*, 99: 577, 1954.
210. Deutsch, H. F. and Nichol, J. C. Biophysical studies of blood plasma proteins. Fractionation studies of normal and immune horse serum. *J. Biol. Chem.*, 176: 797, 1948.
211. Kunkel, H. G. and Tiselius, A. Electrophoresis of proteins on filter paper. *J. Gen. Physiol.*, 35: 89, 1951.
212. Durrum, E. L. Two-dimensional electrophoresis and ionophoresis. *J. Colloid Sc.*, 6: 274, 1951.
213. Block, R. J., Durrum, E. J. and Zweig, G. A Manual of Paper Chromatography and Paper Electrophoresis. New York, 1955. Academic Press, Inc.
214. Kunkel, H. G. and Slater, R. J. Lipoprotein and patterns of serum obtained by zone electrophoresis. *J. Clin. Investigation*, 31: 677, 1952.
215. Kunkel, H. G. and Slater, R. J. Zone electrophoresis in a starch supporting medium. *Proc. Soc. Exper. Biol. & Med.*, 80: 42, 1952.
216. Tyrrell, D. A. J. Separation of inhibitors of agglutination and specific antibodies for influenza viruses by starch zone electrophoresis. *J. Immunol.*, 72: 494, 1954.
217. Sehon, A. H., Kaye, M., McGarry, E. and Rose, B. Localization of an insulin neutralizing factor by zone electrophoresis in a serum of an insulin-resistant patient. *J. Lab. Clin. Med.*, 45: 765, 1955.
218. Payne, R. and Deming, Q. B. Electrophoretic mobility in paper of isoagglutinins. *J. Immunol.*, 73: 81, 1954.
219. Kuhns, W. J. Immunological properties of a form of non-precipitating diphtheria antitoxin which does not sensitize human skin. Studies of its relation to the inhibition reaction. *J. Immunol.*, 75: 105, 1955.
220. Kirkwood, J. G. Suggestions for a new method of fractionation of proteins by electrophoresis convection. *J. Chem. Physics.*, 9: 878, 1941.
221. Cann, J. R., Kirkwood, J. G., Brown, R. A. and Plescia, O. The fractionation of proteins by electrophoresis convection. An improved apparatus and its use in fractionating diphtheria antitoxin. *J. Am. Chem. Soc.*, 71: 1003, 1949.
222. Cann, J. R. The fractionation of gamma globulin by electrophoresis convection. *J. Am. Chem. Soc.*, 71: 2686, 1949.
223. Cann, J. R., Loveless, M. H. Distribution of sensitizing antibody in human serum proteins fractionated by electrophoresis convection. *J. Immunol.*, 72: 270, 1954.
224. Loveless, M. Distribution of "blocking" antibody in human serum proteins fractionated by electrophoresis convection. *J. Immunol.*, 74: 329, 1955.
225. Cann, J. R., Brown, R. A., Gadusek, C., Kirkwood, J. and Sturgeon, P. Fractionation of

Rh antiserum by electrophoresis-conviction. *J. Immunol.*, 66: 137, 1951.

226. Grabar, P. and Williams, C. A., Jr. Méthode permettant l'étude conjuguée de propriétés electrophorétiques et immunochimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim. & biophys. acta*, 10: 193, 1953.
227. Williams, C. A., Jr. and Grabar, P. Immunoelectrophoretic studies on serum proteins. II. Immune sera: antibody distribution. *J. Immunol.*, 74: 397, 1955.
228. Williams, C. A., Jr. and Grabar, P. Immunoelectrophoretic studies on serum proteins. III. Human gamma globulin. *J. Immunol.*, 74: 404, 1955.
229. Porter, R. R. and Humphrey, J. H. The change of antibody character during immunization as judged by partition chromatography. Abstract, Proceedings of the Third International Congress of Biochemistry, Brussels, Belgium, August 1–6, 1955.
230. Isliker, H. C. Purification of antibodies by means of antigens linked to ion exchange resins. *Ann. New York Acad. Sc.*, 57: 225, 1953.
231. Parfentjev, I. U. S. Patent 2,065,196 (1936).
232. (*a*) Pope, C. G. Action of proteolytic enzymes on antitoxins and proteins in immune sera. Heat denaturation after partial enzyme action. *Brit. J. Exper. Path.*, 20: 201, 1939; (*b*) Disaggregation of proteins by enzymes. *Brit. J. Exper. Path.*, 19: 245, 1938; (*c*) Action of proteolytic enzymes on antitoxins and proteins in immune sera. True digestion of proteins. *Brit. J. Exper. Path.*, 20: 132, 1939.
233. Petermann, M. L. and Pappenheimer, A. M., Jr. The ultracentrifugal analysis of diphtheria proteins. *J. Phys. Chem.*, 45: 1, 1941.
234. Peterman, M. L. The splitting of human gamma globulin antibodies by papain and bromelin. *J. Am. Chem. Soc.*, 68: 106, 1946.
235. van der Scheer, J., Lagsdin, J. B. and Wyckoff, R. W. G. Electrophoretic and ultracentrifugal analyses of antipneumococcal horse sera. *J. Immunol.*, 41: 209, 1941.
236. Petermann, M. L. and Pappenheimer, A. M., Jr. Action of crystalline pepsin on horse antipneumococcus antibody. *Science*, 93: 458, 1941.
237. Porter, R. R. The formation of a specific inhibitor by hydrolysis of rabbit antiovalbumin. *Biochem. J.*, 46: 479, 1950.
238. Sturgeon, P. The nature of "complete" Rh antisera as revealed by antiglobulin augmentation. *J. Immunol.*, 73: 212, 1954.
239. Hartley, P. Role of ether-soluble constituents of serum in certain serological reactions. *Brit. J. Exper. Path.*, 6: 180, 1925.
240. Harkins, W. D., Fourt, L., and Fourt, P. Immunochemistry of catalase. II. Activity in multilayers. *J. Biol. Chem.*, 132: 111, 1940.
241. Rosenheim, O. *Biochem. J.*, 31: 54, 1937.
242. Loveless, M. H. Immunological studies of pollinosis. I. The presence of two antibodies related to the same pollen antigen in the serum of treated hay fever patients. *J. Immunol.*, 38: 25, 1940.
243. Boyd, W. C. Effect of high pressures on hemagglutinating antibodies. *J. Exper. Med.*, 83: 401, 1946.
244. Tyler, A. Anaphylactic properties of photo-oxidized rabbit antisera containing "univalent" antibodies. *J. Immunol.*, 51: 329, 1945.
245. Orlans, E. S. The effects of photo-oxidation of antibodies. *Brit. J. Exper. Path.*, 34: 164, 1953.
246. Kleczowski, A. Inactivation of antibodies by ultraviolet radiation. *Brit. J. Exper. Path.*, 35: 402, 1954.
247. Hanan, R. The effect of ultra-violet irradiation on the reactivity of antibody. I. *J. Immunol.*, 69: 41: 1952.
248. Marrack, J. R. and Orlans, E. S. The effect of acetylation of amino groups on the reactions of antigens and antibodies. *Brit. J. Exper. Path.*, 35: 389, 1954.
249. Eisen, H. and Keston, A. S. The immunological reactivity of bovine serum albumin labelled with trace amounts of radioactive iodine. *J. Immunol.*, 63: 71, 1949.
250. Knox, W. C. and Endicott, F. C. I^{131} as an antigen label in the circulating serum of non-immune rabbits. *J. Immunol.*, 65: 523, 1950.
251. Masouredis, S. P. The use of I^{131} trace iodination as a technique for labelling proteins. Thesis, University of California, Berkeley, 1951.
252. Dixon, F. The uses of I^{131} in immunologic investigation. *J. Allergy*, 24: 547, 1953.
253. Wormall, A. Isotopes in the study of immunology. *Brit. M. Bull.*, 8: 223, 1952.
254. Pressman, D., Eisen, H., Siegel, M., Fitzgerald, P., Sherman, B. and Silverstein, A. The zone of localization of antibodies. X. The use of radioactive sulfur^{35} as a label for anti-kidney serum. *J. Immunol.*, 65: 559, 1950.
255. Svedberg, T. and Pederson, K. O. The Ultracentrifuge. London, 1940. Oxford University Press.
256. Kabat, E. A. Molecular weight of antibodies. *J. Exper. Med.*, 69: 103, 1939.
257. Davis, B. D., Moore, D. H., Kabat, E. A. and Harris, A. Electrophoretic ultracentrifugal and immunochemical studies on Wassermann antibody. *J. Immunol.*, 50: 1, 1945.
258. Pederson, K. O. Ultracentrifugal Studies on Serum and Serum Fractions. Uppsala, Sweden, 1945. Almquist & Wiksell.
259. Filitti-Wurmser, S., Aubel-Lesure, G. and Wurmser, R. Constantes de sedimentation des isoagglutinines β(A,O), β(O,O) et β(A,A). *J. de chim. phys.*, 50: 236, 1953.
260. Heidelberger, M., Treffers, H. P. and Mayer, M. Quantitative theory of precipitin reaction. Egg albumin-antibody reaction in antisera from rabbit and horse. *J. Exper. Med.*, 71: 271, 1940.
261. Mayer, M. and Heidelberger, M. Velocity of combination of antibody with specific polysaccharides of pneumococcus. *J. Biol. Chem.*, 143: 567, 1942.
262. Boyd, W. C. Assay of blood grouping sera. Variation in reactivity of cells of different individuals belonging to group A and AB. *Ann. New York Acad. Sc.*, 46: 927, 1946.

263. Campbell, D. H., Luescher, E. and Lerman, L. S. Immunologic adsorbents. I. Isolation of antibody by means of a cellulose-protein antigen. *Proc. Nat. Acad. Sc.*, 37: 575, 1951.

264. Sumner, J. and Kirk, J. S. Über die chemische Natur der Urease. *Ztschr. f. physiol. Chem.*, 205: 219, 1932.

265. Heidelberger, M. and Kendall, F. E. Quantitative studies on antibody purification. Dissociation of precipitates formed by pneumococcus specific polysaccharides and homologous antibodies. *J. Exper. Med.*, 64: 161, 1936.

266. Heidelberger, M., Kendall, F. E. and Teorell, T. Quantitative studies on precipitin reaction. Effect of salts on reaction. *J. Exper. Med.*, 63: 819, 1936.

267. Sternberger, L. A. and Pressman, D. General method for specific purification of antiprotein antibodies. *J. Immunol.*, 65: 65, 1950.

268. Karush, F. Preparation and specific interactions of purified anti-hapten antibody. *Federation Proc.*, 12: 448, 1953.

269. Kuhns, W. J. and Dixon, F. J. Immunochemical studies of complexes formed between I^{131} labelled diphtheria toxin and human antitoxin. Paper presented at the Third International Congress of Biochemistry, Brussels, Belgium, August 1–6, 1955.

270. Pappenheimer, A. M., Jr. The valency of antibodies. In: Pappenheimer, A. M., Jr. The Nature and Significance of Antibody Response. New York, 1953. Columbia University Press.

271. Brambell, W. F. R., Hemmings, W. A. and Henderson, M. Antibodies and Embryos. London, 1951. University of London, The Athlome Press.

272. Florman, A. L., Schick, B. and Scalettar, H. E. Placental transmission of mumps and streptococcus MG antibodies. *Proc. Soc. Exper. Biol. & Med.*, 78: 126, 1951.

273. Adamson, C. A., Lörgren, S. and Mälmnas, C. Antibodies in mothers and newborn infants. *Scandinav. J. Clin. & Lab. Invest.*, 3: 52, 1951.

274. Murray, J., Colman, R. M. and Lepine, A. Transmission of staphylococcal antitoxin from mother to child. *Lancet*, 14: 259, 1950.

275. (*a*) Osborn, J., Dancis, J. and Julia, J. Studies of the immunology of the newborn infant. I. Age and antibody production. *Pediatrics*, 9: 736, 1952; (*b*) Osborn, J., Dancis, J. and Julia, J. Studies of the immunology of the newborn infant. II. Interference with active immunization by passive transplacental circulating antibody. *Pediatrics*, 10: 382, 1952; (*c*) Osborn, J., Julia, J. and Rosenberg, B. Studies of the immunology of the newborn infant. III. Permeability of the placenta to maternal antibody during fetal life. *Pediatrics*, 10: 390, 1952.

276. Mollison, P. L. Blood Transfusion in Clinical Medicine. Oxford, 1951. Blackwell Scientific Publications.

277. Longsworth, L. G. Curtis, R. M. and Pembroke, R. H., Jr. The electrophoretic analysis of maternal and fetal plasmas and sera. *J. Clin. Investigation*, 24: 46, 1945.

278. Bell, S. D. and Eriksson, Z. Studies in transmission of sensitization from mother to child in human beings. Transfer of skin sensitizing antibodies. *J. Immunol.*, 20: 447, 1931.

279. Zohn, B. Placental transmission of hypersensitiveness to ascaris lumbricoides actively induced in the pregnant woman. *Am. J. Dis. Child.*, 57: 1067, 1939.

280. Sherman, W. B., Hampton, S. F. and Cooke, R. A. The placental transmission of antibodies in the skin sensitive type of human allergy. *J. Exper. Med.*, 72: 611, 1940.

281. Wiener, A. S. Rh factor in immunological reactions. *Ann. Allergy*, 6: 293, 1948.

282. Broman, B. Incompatibility of blood. *Acta paediat.*, 36: 585, 1949.

283. Timmerman. Cited by Colman, R. M. and Murray, J. In: The transfer of antibodies across the placenta. *Endeavour*, 10: 27, 1951.

284. Hartley, P. The effect of peptic digestion on the properties of diphtheria antitoxin. *Proc. Roy. Soc., London, s. B.*, 138: 499, 1951.

Clinico-pathologic Conference

Central Nervous System Disease Characterized by Low Spinal Fluid Sugar

STENOGRAPHIC reports, edited by Amoz I. Chernoff, M.D. and W. Stanley Hartroft, M.D., of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient (No. 254660), a fifty-four year old white farmer, was admitted to the Medical Service of Barnes Hospital on May 21, 1955, complaining of nausea, vomiting, headache and dizziness.

The patient considered himself in good health until three months prior to admission when he noticed the onset of occasional frontal headaches, rare dizzy spells characterized by spinning sensations, and staggering gait, more to the left than to the right. Two weeks later he began having right lower quadrant pain and fever. A diagnosis of acute appendicitis was made and confirmed at operation when an acutely inflamed appendix was removed. On the third postoperative day an incisional abscess developed which subsequently healed. Following the operation he had almost constant frontal "expanding" headaches which varied in intensity and were partially relieved for short periods by aspirin. During the month prior to admission he became progressively weaker, necessitating his staying in bed most of the time. Diplopia, blurring of vision and transient episodes of mental confusion appeared. Nausea and vomiting progressed to the point where he could only retain liquids. A gastrointestinal fluoroscopic examination done before admission to Barnes Hospital was said to be negative. He lost 30 pounds during the three months of his illness.

Three years prior to admission he had a vague illness characterized by drowsiness, weakness, afternoon fever, night sweats and a 70 pound weight loss, but did not consult a physician. Over a period of several months the symptoms gradually subsided, and he slowly regained his weight. One year prior to admission he made a trip through the San Joaquin Valley of California where he was exposed to a great deal of dust. He had no symptoms during or following the trip. Six months prior to admission he struck his head without loss of consciousness or apparent residual effects.

Physical examination at the time of entry revealed his temperature to be 37.8°C.; pulse, 54; respirations, 14 and blood pressure, 120/70. The patient was a well developed, moderately obese, chronically ill man who was drowsy but well oriented and capable of answering questions. The skin was clear and no lymph nodes were felt. There was minimal tenderness over the frontal sinuses. The neck was moderately rigid with pain in the cervical and thoracic spine area on flexion. The left pupil was larger than the right pupil; both reacted to light and accommodation. A persistent horizontal nystagmus on lateral gaze with the fast component in the direction of gaze was noted. There was no diplopia. Gross confrontation visual fields were intact. The optic fundi appeared normal. Examination of the ears, nose, throat, lungs, heart and abdomen was not remarkable. A slight jarring pain was produced by fist percussion over the spine. A supratesticular mass which transmitted light was felt on the right. Neurologic examination revealed the mental status, cranial nerves, sensory perception, and superficial and deep tendon reflexes to be normal. A 3+ jaw jerk was present. There was generalized weakness, most marked in the right hand. The finger-to-nose test was performed well except for jerky movements and a terminal gross tremor bilaterally. He stood and walked with a wide base, staggering to the left. The Romberg sign was positive, the patient falling to the left.

The laboratory data were as follows: blood

counts: hemoglobin, 13.0 gm. per cent; white blood count, 5,850; differential count: eosinophils, 1; bands, 2; segmented forms, 58; lymphocytes, 34; monocytes, 5. The red blood cells and platelets appeared normal. Urinalysis: specific gravity, 1.018; reaction, 5.5; protein, negative; sugar, negative; microscopic examination revealed rare fine granular casts, 1 to 3 white blood cells and occasional red blood cells per high power field. Stool: guaiac-negative. Blood cardiolipin: negative. Blood chemical determinations: non-protein nitrogen, 24 mg. per cent; fasting blood sugar, 75 mg. per cent; carbon dioxide combining power, 30.0 mEq./L.; sodium, 136 mEq./L.; potassium, 4.0 mEq./L.; total protein, 6.3 gm. per cent; albumin, 4.4 gm. per cent; globulin, 1.9 gm. per cent; cephalin cholesterol flocculation, negative; thymol turbidity, 2.6 units; alkaline phosphatase, 2.2 Bodansky units. Lumbar puncture: initial pressure, 80 mm. of water; final pressure, 30 mm. of water after removal of 10 ml. of fluid which appeared xanthochromic; cells with acid, 170 per cu. mm.; cells without acid, 160 per cu. mm.—all mononuclear cells; protein, 538 mg. per cent; sugar, 17 mg. per cent; chloride, 120 mEq./L.; colloidal gold, 0000000000; Wassermann, negative. Smear of the spinal fluid sediment stained with Gram's stain was negative. No acid-fast bacilli were seen. India ink preparations failed to reveal Torula organisms. Routine and fungus cultures were negative, and at the time of the patient's death the cultures for tuberculosis revealed no growth. Sputum cultures and smears were negative for pathogens. Skin tests: first and second strength P. P. D., negative; old tuberculin, 1:10, slightly positive; histoplasmin, positive; coccidiodin, negative. Roentgenograms: The chest film was interpreted by the roentgenologist as showing pulmonary emphysema, (?) emphysematous bullae left lung, (?) pneumonitis left lower lobe, and primary calcified complex on the right. Roentgenogram of the skull revealed a normal skull and sella turcica, non-visualized pineal body and calcification of the falx. Films of the dorsal and lumbar spine were interpreted as showing "hypertrophic degenerative arthritis of the dorsal and lumbar spine." Electrocardiogram: "sinus tachycardia with marked clockwise rotation." The electroencephalogram showed a slow dysrhythmia without focal or lateralizing signs. The interpretation was "consistent with organic contributory basis. With this type of record consider electrolyte imbalance."

On the day following admission the patient was definitely confused. Nausea and vomiting were partially controlled with chlorpromazine. He refused to eat, however, and his food and fluid intake were supplemented by intravenous fluids containing glucose, saline, potassium and vitamins. Antituberculous therapy consisting of isonicotinic acid hydrazide, 500 mg. per day intramuscularly, sodium para-aminosalicylate, 15 gm. intravenously per day, and streptomycin, 1 gm. twice a day, was started on the fourth hospital day. To facilitate the intravenous administration of fluids and medications, a polyethylene catheter was inserted into a vein in the left leg. By the sixth hospital day the patient was in a constant semi-stuporous state and totally incontinent of urine. Neurologic examination revealed a bilateral Gordon reflex, hyperactive knee jerk and ankle jerk on the left, constricted pupils with minimal inequality, and nystagmus on forward gaze with the fast component to the left. On this day the patient spiked a fever to 38.8°C. but the temperature returned to normal the same day and remained normal until the fourteenth hospital day. During this time his state of consciousness fluctuated as did his neurologic signs. Increased resistance to passive stretch developed, with hyperactive reflexes on the right side of his body and frequent muscular twitches. Several repeat lumbar punctures were performed; the findings are listed in Table 1. The spinal fluid was always noted to be xanthochromic and bacteriologic study was consistently negative. Because of fever and signs of thrombophlebitis in the left leg, the polyethylene catheter was removed. A blood culture taken at this time revealed Micrococcus var. aureus with a density of approximately five colonies per cubic centimeter. He was given penicillin, 400,000 units intramuscularly, every four hours. In addition to the urinary incontinence, one episode of acute urinary retention occurred and a Foley catheter was inserted. Because of the persistent fever the finding of a confluent growth of organisms on urine culture and a white blood count of 18,600 with a shift to the left, tetracycline, 100 mg. every four hours intramuscularly, was administered. The patient's condition slowly deteriorated. On the twentieth hospital day he was started on pyrizinamide, 500 mg. every four hours by mouth,

TABLE 1
SPINAL FLUID FINDINGS

Hospital Day	Initial Pressure (mm. of water)	Spinal Fluid Removed (ml.)	Final Pressure (mm. of water)	Cell Count (all mononuclears) (cu. mm.)	Protein (mg. %)	Sugar (mg. %)	Chloride (mEq./L.)
1	80	10	30	160	538	17	120
3	80	15	70	300	518	11	121
8	70	..	...	260	440	13	120
13	120	10	110	90	700	4	119
19	250	15	190	40	...	..	...
23	110	8	65	32	600	13	117
27	90	10	80	137	596	23	118

and the sodium para-aminosalicylate was discontinued. A neurosurgical consultant saw the patient because of the possibility that a space-occupying intracranial lesion was causing the right hemiparesis. He did not think that ventriculograms were indicated. The optic discs at this time were definitely more blurred, and most observers thought that papilledema was present. On the twenty-fourth hospital day the patient's fever began to rise slowly and he became completely comatose. Erythromycin was added to the regimen of antibiotics. Blood cultures were sterile. During the last five days of life, the patient rapidly deteriorated; his temperature rose to 41°C. on the day before death and he went into shock. Attempts to maintain the blood pressure with vasoxyl,® norepinephrine and hydrocortisone were of no avail, and the patient died on June 18, 1955, the twenty-ninth hospital day. A few minutes before death his temperature was 42°C.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: I believe it would be worth while to summarize this patient's symptoms and pertinent neurologic findings. You will recall that on admission to the hospital the patient gave a history of intermittent vomiting, expanding-type headaches, dizziness and a staggering gait of about three months' duration. During this three-month period he also had lost 30 pounds. In addition, drowsiness, weakness, afternoon fever and night sweats had been present for a period of several months. Two weeks after the onset of the illness symptoms suggestive of acute appendicitis developed, and this diagnosis was presumably confirmed at operation. For one month prior to admission to the hospital he had progressive weakness, diplopia, blurring of vision and transient episodes of mental confusion. Progressive nausea and vomiting had been present for a period of two or three weeks. Three years prior to his admission he recovered spontaneously over a period of several months from an illness characterized by weakness, fever, night sweats and weight loss, no diagnosis ever having been established. Physical findings included a low grade fever and a pulse rate which was in proportion to the temperature. You will note in the protocol that the pulse rate was 54. On the patient's admission to the hospital the intern and assistant resident noted a definite bradycardia, but it should be emphasized that this was not observed at any other time. There were no significant physical findings other than those referable to the neurologic system. On admission, the patient was lethargic and had generalized weakness. The left pupil was larger than the right. There was persistent horizontal nystagmus in all directions of gaze except straight forward. The jaw jerk reflex was very active. There were jerky movements on the finger-to-nose test with either hand, with a terminal gross tremor. The patient stood and walked with a wide base and staggered to the

left. The Romberg sign was positive, the patient tending to fall to the left. On the sixth hospital day he was semi-stuporous, completely incontinent of urine and the stiffness of his neck, which was noted on admission, had become more marked. A bilateral Gordon sign was noted. The knee jerks and ankle jerks are said to have been questionably more active on the left than on the right, but the examiner apparently placed little significance in this finding. The pupils were constricted with minimal inequality. There was nystagmus even on forward gaze with the fast component to the left. Progressively, after the sixth day, increased resistance to passive stretch, hyperactive reflexes on the right side of the body and frequent muscle twitches developed. On the twentieth hospital day the optic discs were blurred. By the twenty-fourth hospital day the fever, which had been ranging between approximately 37.5° and 38.5°C., began to rise progressively and deepening coma developed. By the twenty-seventh hospital day he was in shock which could not be controlled with nor-epinephrine. On the twenty-eighth day the patient died, the temperature having reached the level of 41.6°C., or 107°F., immediately prior to death. Dr. Seaman, would you now review the x-ray films?

Dr. William B. Seaman: Roentgenograms of the skull and chest were taken about two days after the patient entered the hospital. The skull examination was essentially negative. The sella showed no sign of increased intracranial pressure; no intracranial calcifications were seen. On the lateral view a very faint collection of calcium was seen in the region of the pineal. We could not be sure that this was the pineal gland, but if it were, it lay within the normal position. The calcification of the falx seen in the postero-anterior view appeared to be of no clinical significance. The chest film taken on the same day showed a primary complex in the right lower lobe and calcified right hilar nodes. The original report raised the question of pneumonitis in the left lower lobe; personally, I think that it was more likely due to incomplete aeration, because the diaphragms were a little higher than normal. A few small focal areas of atelectasis were noted in the left lower lobe; this abnormality again is usually associated with elevation of the diaphragm. I do not believe there was anything clinically significant in the chest film. Examination of the dorsal and lumbar spine was essentially negative, except for the presence of rather marked hypertrophic changes, but no evidence of tumor or infection. The last x-ray films were obtained on June 15th, about three days prior to the patient's death. Examination of the chest with the patient in the supine position again revealed the calcified primary focus, but essentially no significant findings.

Dr. Reinhard: Dr. Levy, let us first concentrate our attention on the symptoms and neurologic findings. I wonder if you would comment on those neurologic findings which seem to be of significance to you, and tell us how you would interpret them. What thoughts would go through your mind as a neurologist on first seeing this patient with no laboratory data or spinal fluid examination available?

Dr. Irwin Levy: I think we would have to interpret the first symptoms of vomiting, headache and dizziness as either evidence of an intracranial space-occupying lesion or of meningeal involvement. I do not believe we could differentiate tumor versus meningitis from these three symptoms alone. If we consider tumor, I think we would have to say that the early symptoms of vomiting, headache and dizziness would, perhaps, be more suggestive of posterior fossa disease than of supratentorial disease. To go on to the question of the staggering gait, later described as having a wide base, we would suspect some involvement of the vestibulo-cerebellar system, and from further data one would be apt to involve the cerebellum itself. As far as the weight loss is concerned, this could be due either to chronic infection or to tumor, with the associated headaches and vomiting contributing to a disturbed nutritional state. The acute appendicitis was probably coincidental although it is true that one occasionally finds infections with symptoms of acute abdominal conditions complicating intracranial disease, which have been misinterpreted as acute surgical conditions of the abdomen.

Dr. Reinhard: It is also true that in appendiceal abscess, brain abscess may be a complication. Is that correct?

Dr. Levy: Yes.

Dr. Reinhard: But in this case the chronology is not correct for that.

Dr. Levy: When we come to the diplopia and blurring of vision, I think we must say that in all probability there was involvement of the occulomotor pathways, in view of the patient's other symptoms in the extra-axial or sub-

arachnoid space. This could either be by distortion, as for example with a space-occupying lesion, or by cellular invasion or inflammation of the meninges. So again we are faced with the two possibilities. The progressive nature of the disease would fit with either a progressive chronic inflammation of the meninges, tumor involvement of the meninges or an expanding space-occupying lesion of the intracranial cavity. I do not think we can use that for differential diagnosis. Regarding signs and symptoms, if we were to consider that the slow pulse was significant, the first thing the neurologist thinks of is the possibility of a brain abscess with a disproportionately slow pulse. The bradycardia was not verified on subsequent examinations, so that we would be apt to overlook the initial slow pulse. Examination indicated that the patient was lethargic and had generalized weakness. These manifestations we would interpret as due to one of two things, which may actually go together: either brain stem involvement from the pathologic process, such as meningitis, with interference of the blood supply to the brain stem, or interference with cortical activity, as for example in confluent meningitis or diffuse involvement of the meninges by tumor. The fact that the left pupil was involved, and at one time noted to be dilated, would suggest that there was some third nerve involvement, which would go along with the diplopia recorded in the history. This could take place anywhere from the interpeduncular space forward through the cavernous sinus. The nystagmus, along with the tendency to fall to the left in the Romberg test, would suggest primarily left cerebellar involvement. The patient also had a rather gross tremor which is somewhat difficult to interpret because it could occur with some depression in consciousness and confusion as well as with cerebellar involvement. The fact that the patient had a stiff neck would lead us to lean more toward meningeal involvement rather than toward a tumor as such, so that we would begin to believe that there may have been both considerable cerebellar involvement and meningeal involvement. The pyramidal tract signs, first apparent on the left and soon thereafter on the right, would suggest that obstructive hydrocephalus was beginning as a result of obstruction of the foramens of Luschka and Magendie as a terminal event resulting from invasion of the meninges.

DR. REINHARD: Thank you very much. Dr. O'Leary, will you tell us what you think about the pressure changes before and after removal of spinal fluid, in terms of a space-occupying lesion. Are these pressure changes on the removal of fluid for or against the diagnosis of a space-occupying lesion?

DR. JAMES L. O'LEARY: As I look over this series of pressure changes I cannot see them as consistent with a diagnosis of neoplasm. We start out on the first hospital day with an initial pressure of 80 and a closing pressure of 30 after removal of 10 cc. As I go down through the dynamics of the succeeding punctures I do not see any significant changes from the initial values reported, so I would conclude that the initial and closing pressures do not give positive support for the diagnosis of a space-occupying lesion, although we all know that you could have a tumor with such pressures.

DR. REINHARD: Considering everything we have discussed so far—the symptoms, the clinical findings and the spinal fluid findings—do you think this patient had a space-occupying lesion?

DR. O'LEARY: I do not think I could say whether or not this patient had a space-occupying lesion. Dr. Levy has covered the other diagnostic possibilities extensively, but I do not believe, with the information we have available, that we can eliminate a space-occupying lesion.

DR. REINHARD: Dr. Rosenbaum, on the nineteenth hospital day you wrote a note suggesting that perhaps ventriculograms and careful examination of the ventricular fluid might be indicated. At this time the patient's condition was getting steadily worse in spite of intensive antitubercular therapy. In retrospect, do you think this procedure should have been carried on?

DR. HERBERT ROSENBAUM: At that particular point in the course I believed that a tumor was most likely and that the air studies could shed some light on this possibility. This procedure is particularly helpful in instances of posterior fossa tumors and I believe that a cerebellar pontine angle tumor is the most likely diagnosis today.

DR. REINHARD: Dr. Schwartz, the neurosurgical consultant stated that he did not think the ventriculograms were indicated. How do you feel about this? Would such studies have been of any help?

DR. HENRY G. SCHWARTZ: I do not believe ventriculography would have helped in this

case. Although some of the symptoms manifested by the patient were consistent with a cerebellar tumor associated with increased intracranial pressure, this man actually had no evidence of increased intracranial pressure at any time. The question of ventriculography arose at the time when there appeared to be blurring of the optic discs, a finding which developed long after it had been noted that he had pupillary inequality. It is difficult for me to conceive of pupillary inequality due to a posterior fossa tumor without fairly definite signs of increased intracranial pressure coming on long before the nineteenth or the twentieth day. It was our belief that this patient presented a diffuse involvement of the nervous system. He was obtunded out of all proportion to the level of spinal fluid pressure. He did have cerebellar and vestibular signs, so that we believed he had a diffuse lesion involving the middle fossa as well as the posterior fossa and possibly even the convexity in the frontal region. We, therefore, did not believe that ventriculography was necessary.

DR. REINHARD: Dr. Schwartz, the possibility was mentioned several times in the chart that the patient might have tuberculous meningitis with a subarachnoid block, due to thick exudate. Would you discuss the evidence here for and against a subarachnoid block.

DR. SCHWARTZ: There was no evidence of that being present in this case. Apparently there was a free flow of spinal fluid.

DR. REINHARD: I believe the idea was that with evidence of optic disc blurring and a normal spinal fluid pressure there could have been a block at the foramen, for example.

DR. SCHWARTZ: In such cases one would expect that the optic disc would be somewhat more than blurred, Dr. Reinhard. I think there would be no question about papilledema being present. I do not believe that we have the evidence for obstruction to the flow of spinal fluid between the posterior fossa and the spinal canal.

DR. REINHARD: I certainly agree with that. Dr. Levy, we have already mentioned the pressure readings. Would you tell us how you would interpret the other findings listed in Table 1?

DR. LEVY: The xanthochromia is entirely consistent with the elevation of protein. In relationship to the cells it would be interesting, from the standpoint of possible tumor involving the meninges, to know whether cell studies had been made for that purpose. Apparently the cells were all counted as mononuclears. Such a pleocytosis could be indicative of a chronic infectious process. It would be consistent with tuberculosis or one of the yeast infections. It is also consistent with a diagnosis of tumor cells in the spinal fluid. The protein is certainly compatible with chronic meningitis. It is so high that it tends to lead one to consider meningeal metastases with tumor a bit more seriously. Such very high protein levels are seen in patients with an acoustic neuroma; however, in view of the rest of the picture, I would be more inclined to suggest that it was related to meningeal involvement rather than to an acoustic tumor.

DR. REINHARD: I would like to ask Dr. Harford if he would comment on the repeated failure to demonstrate acid-fast bacilli either by direct smear or in cultures. Is this weak or strong evidence against tuberculous meningitis? You will recall that only the first two spinal fluid examinations were done prior to the onset of antituberculous therapy. Does this help us very much?

DR. CARL G. HARFORD: It is not conclusive, but I think it has to be considered as being against the diagnosis of tuberculous meningitis.

DR. REINHARD: I would like to consider in some detail the very interesting low spinal fluid sugar. Such a finding is generally considered to be strong evidence in favor of infectious meningitis, either pyogenic meningitis or tuberculous meningitis. Dr. Harford, you have done some experimental work on this subject, and I wonder if you would discuss the mechanism by which the spinal fluid sugar is reduced in infectious meningitis.

DR. HARFORD: Several years ago Dr. Sidney Goldring tried to elucidate the mechanism.[1] He injected either serum or penicillin intracysternally into dogs to produce aseptic meningitis and then measured the spinal fluid sugars on fluid removed from such dogs. In *in vitro* experiments he determined how much sugar was utilized by the leukocytes in the spinal fluid. He found that the leukocytes utilized a small amount of sugar, but apparently not enough to cause a lowering of the level of the spinal fluid glucose. He, therefore, concluded

[1] GOLDRING, S. and HARFORD, C. G. Effect of leucocytes and bacteria on glucose content of the cerebrospinal fluid in meningitis. *Proc. Soc. Exper. Biol. & Med.*, 75: 669, 1950.

that there must have been some type of replenishment mechanism which enabled the spinal fluid sugar to be normal while the *in vitro* evidence indicated there was some utilization. Similar experiments using Type III pneumococci indicated that very large numbers of rapidly growing organisms had to be present in the spinal fluid to get about as much utilization of sugar as was observed with large numbers of leukocytes. On the basis of these data he came to the conclusion that it was difficult to explain the low spinal fluid sugar in bacterial meningitis solely on the basis of utilization of sugar either by bacteria or by leukocytes and that there must be some other factor operating. It is already known that lymphocytes and mononuclear cells utilize less sugar than polymorphonuclear leukocytes. Furthermore, tubercle bacilli utilize less sugar than do rapidly growing bacteria, so it seems very difficult to account for the low spinal fluid sugars in tuberculous meningitis on the basis of utilization. Another possibility must be considered, namely, that there might be some interference with the transport of glucose into the spinal fluid. Subsequently a study was reported in which normal children and children with tuberculous meningitis were given intravenous infusions of glucose.[2] Glucose determinations on the spinal fluid obtained both by cysternal and lumbar punctures were carried out, and it was found in a number of instances that there was a decreased delivery of glucose to the spinal fluid in the patients who had active tuberculous meningitis. This evidence suggests that in tuberculous meningitis there is some failure in the transport of glucose to the spinal fluid in addition to the other factors which probably play some role. It is known that tumor cells utilize glucose more rapidly than normal cells. Perhaps this might also be a contributing factor.

DR. REINHARD: If there were a very extensive invasion of the meninges by tumor cells might there not also be some interference with the transport of glucose across this invaded membrane?

DR. HARFORD: Yes.

DR. REINHARD: In 1953 Dr. Berg published a review of the literature on hypoglycorrhachia (or low sugar content in the subarachnoid space) of non-infectious origin, and reported three additional cases.[3] This report seemed so pertinent to this presentation that I have taken the liberty of asking Dr. Berg to discuss this problem.

DR. LEONARD BERG: It is certainly true that not all instances of lowered spinal fluid sugar are due to infection in the meninges. There are a number of non-infectious causes, the most common of which seems to be operative when neoplasms metastasize diffusely to the subarachnoid space. These neoplasms may be carcinomas, tumors of glial origin, sarcomas, lymphomas; indeed, it seems that almost any tumor that has metastasized diffusely to the subarachnoid space may be accompanied by a decreased content of sugar in the spinal fluid. We were able to collect from the literature approximately sixty cases of diffuse meningeal metastases in which the results of spinal fluid sugar determinations were low. Since then there have been another six or eight cases published. It was apparent that the sugar content is below normal in the majority of these cases. Since there is some question about the selection of case material for publication I believe that precise figures as to the incidence of low spinal fluid sugar cannot be given, but certainly many cases with diffuse meningeal metastases from neoplasms of one sort or another may present with low spinal fluid sugars. In the group of diffuse meningeal metastases from carcinoma, gastric neoplasms are the most frequent primary tumors.

DR. REINHARD: Do you happen to know whether any of these patients with carcinoma of the stomach had extensive spread to the meninges of their carcinoma, causing a low spinal fluid sugar without any signs pointing to the primary tumor, or were these all cases of far-advanced carcinomatosis?

DR. BERG: No. Most of these patients presented early with neurologic syndromes. Indeed, in some of these cases, when the investigators were already aware of the possibility and careful search by radiologic technics were made for primary sites, no tumors were found. At autopsy the primary tumor might be the size of a pea in a bronchus or a very small malignant

[2] SIFONTES, J. E., WILLIAMS, R. D. B., LINCOLN, E. M. and CLEMONS, H. Observations on the effect of induced hyperglycemia on the glucose content of the cerebrospinal fluid in patients with tuberculous meningitis. *Am. Rev. Tuberc.*, 67: 732, 1953.

[3] BERG, L. Hypoglycorrhachia of non-infectious origin: diffuse meningeal neoplasia. *Neurology*, 3: 811, 1953.

ulcer in the stomach, yet the majority of these cases did present with neurologic syndromes. The spinal fluid sugar content was noted to be quite variable: some normal values, borderline values, others quite low. In one case reported from the Mayo Clinic no sugar was demonstrable in the spinal fluid by quantitative testing on two separate occasions. Furthermore, there is a striking lack of correlation between the spinal fluid sugar content and either the number of cells or the amount of protein present, although in the majority of the cases the spinal fluid protein is elevated to a moderate or considerable degree. Other tumors that spread diffusely through the meninges, such as primary mesodermal tumors arising from the meninges, lymphomas, melanomas and the like, may also present with abnormally small amounts of sugar in the spinal fluid, although there are some with perfectly normal sugar contents. Again, the correlation between the amount of sugar and pleocytosis and the amount of protein is not good. Spinal fluid chlorides were recorded in some of these cases, and again no correlation with the sugar content was obvious. In essence then, spinal fluid sugar content is frequently low in cases of diffuse meningeal metastases. This finding is poorly correlated with other abnormalities in the spinal fluid. As to mechanisms, if one visualizes the spinal fluid circulating among great masses of tumor cells, it is easy to appreciate that there is ample opportunity for these metabolically active cells to utilize sugar, probably at an increased rate compared with normal cells. The sugar content may therefore be reduced to below normal. In cases of infectious meningitis the observation that lactic acid and pyruvic acid levels in the spinal fluid are increased provides some further evidence that utilization is an important factor. To my knowledge these observations have not been made in spinal fluid or patients with diffuse meningeal metastases.

DR. REINHARD: If this patient does have diffuse neoplastic disease rather than infectious meningitis, it would appear from the data that Dr. Berg has presented in his review of the literature up to 1953, that on a purely statistical basis the most likely type of tumor would be carcinoma of the stomach. If, on the other hand, the patient does not have metastatic carcinoma but rather a primary tumor of the central nervous system, we may then consider those tumors listed in Dr. Berg's review of the literature which indicated that there were five examples of primary meningeal tumors producing the low spinal fluid sugar, four cases of glioblastoma, two of medulloblastoma, two of astrocytoma, one malignant ependymoma and one pinealoma. Dr. Schwartz, are there any other tumors which on theoretical grounds ought to be added to this list, even though examples are not reported in the literature?

DR. SCHWARTZ: One should add to the list the dermoid and epidermoid tumors. Whether or not you want to consider these as primary brain tumors is another question. Certainly they can lie within the folds of the brain, and will cause extensive changes in the meninges. The cases which result in the most severe meningeal reactions are those of the epidermoid group in which cholesterol deposits may be seen in the subarachnoid space, giving a picture of fulminating meningitis.

DR. REINHARD: Is there anything about this case that would tend to be of any help to you in picking out one of these? Could you eliminate any of these?

DR. SCHWARTZ: Statistically, I think one could eliminate medulloblastoma. This patient was a man in the older age group in whom one would not expect to find a medulloblastoma, and yet they do occasionally occcur. The ependymomas would also be less likely to occur in this age group. Malignant ependymomas may be seen in any age group, and our experience has been that they are of the type which does spread diffusely through the nervous system. However, they are more common in younger people than in older ones. The most common glial tumor in the older age group, of course, is the glioblastoma.

DR. REINHARD: How about malignant melanoma? There are three examples in the literature producing this situation. Does this occur in the meninges of the brain without tumor elsewhere?

DR. SCHWARTZ: It can occur, just as one can find isolated Hodgkin's disease in the brain without its occurring elsewhere. Ordinarily there is a history of a preceding lesion, perhaps many years before, in some other part of the body. Malignant melanoma certainly has to be considered, as well as so-called Boeck's sarcoid which can involve the nervous system.

DR. BERG: I would like to say that meningitis secondary to dermoid and epidermoid tumors

is not accompanied by lowered spinal fluid sugar. It is a chemical, aseptic meningitis, and the sugar content remains normal just as it did in Goldring's and Harford's dogs after the intrathecal injection of penicillin or serum.

DR. REINHARD: Dr. Goldman, do you believe that the fact that this patient failed to respond to fairly intensive antituberculous therapy is strong evidence against tuberculous meningitis being the correct diagnosis in this case?

DR. ALFRED GOLDMAN: Yes, I think that is strong evidence against such a diagnosis, except in a patient who is admitted in a terminal state.

DR. REINHARD: The house staff diagnoses were (1) chronic leptomeningitis; (2) tuberculous leptomeningitis, suspected; and (3) unspecified tumor of the meninges, suspected. They were listed in this order. My final diagnosis is malignant tumor extensively involving the meninges in the brain, possibly due to metastatic carcinoma most likely from the stomach; as a second possibility I suggest a primary brain tumor with meningeal involvement.

PATHOLOGIC DISCUSSION

DR. ROBERT C. AHLVIN: Examination of the thoracic and abdominal viscera revealed moderate arteriosclerosis of the aorta and coronary arteries. The heart was slightly dilated and hypertrophied, weighing 420 gm. There was focal fibrosis of the myocardium. The congested lungs were edematous, and there was early bronchopneumonia. There was a slight fibrous thickening of the pia arachnoid over the lumbar cord and cauda equina. Over the cerebral hemispheres there was a similar but less extensive fibrous thickening of the pia arachnoid. The cerebral convolutions were flattened. There was distinct uncal grooving. Coronal sections of the brain revealed the ventricular cavities to be lined by a greyish red membrane which appeared to be hemorrhagic ependymitis. The choroid plexus were grey, flattened and thickened, being adherent to the ventricular walls. The ventricles were slightly dilated. The cerebral tissue adjacent to the ventricular cavities was grey and semi-translucent.

Microscopically the granular lining of the ventricles proved to be a tumor which was made up of two cellular types, the general pattern of which is shown in Figure 1. A small cell characterized by scant cytoplasm and by a darkly staining nucleus resembles a lymphocyte, and the other cell is larger. It has more cytoplasm and a larger nucleus than the small cell and its nucleolus is prominent. (Fig. 2.) Tumors of this architectural pattern, in general, arise from one of three sites: ovary, testis or pineal. (A dysgerminoma could be ruled out since this patient was a man.) The testes were carefully examined and no evidence of a seminoma was found.

In microsections of the wall of the lateral ventricle the ependyma was replaced *in toto* by malignant tumor cells which extended into the adjacent cerebral tissue and often surrounded blood vessels. The aqueduct, third and fourth ventricles were similarly lined by tumor. The pineal gland was not enlarged, but microscopically was involved by neoplastic cells of the two types previously described. Some suggestion of its normal architecture still remained in other portions of the pineal.

Diffuse invasion of the pia arachnoid of the cerebral cortex by rather small numbers of tumor cells of both the types described above is illustrated in Figure 3. A similar infiltration of tumor cells was seen in one of the cranial nerves. (Fig. 4.)

In summary, this case represents that of a man with a pinealoma involving the pineal gland, the ependyma of the lateral, third and fourth ventricles with infiltration of the cranial nerves and metastasis to the pia arachnoid.

DR. SARAH A. LUSE: This case represents a rare manifestation of an equally rare tumor, a diffuse pinealoma in which there is not enlargement, although there is probably involvement, of the pineal gland. One of the larger groups of cases with this type of lesion has been reported from this hospital by Russell and Sachs,[4] who reviewed fifty-eight cases of pinealoma; in eighteen there was diffuse involvement of the ependyma. However, in most of their cases the pineal was enlarged and definitely involved by tumor. Mackay[5] and Russell[6] have both reported cases of diffuse tumor which resembled and were indistinguishable from pinealomas, but in which involvement of the gland by tumor was not even demonstrated. Probably the best interpretation of these lesions is that of Russell,[6] who thought that the pineal, *per se*, need not

[4] RUSSELL, W. O. and SACHS, E. Pinealoma. *Arch. Path.*, 35: 869, 1943.

[5] MACKAY, R. P. Pinealoma of diffuse ependymal origin. *Arch. Neurol. and Psychiat.*, 42: 892, 1939.

[6] RUSSELL, D. The pinealoma; its relationship to teratoma. *J. Path. & Bact.*, 56: 561, 1944.

FIG. 1. Interspersed large and small cells in the ependyma are neoplastic cells arranged in the pattern characteristic of pinealoma.

FIG. 2. Small dark cells are scattered among larger cells which have distinct nucleoli and scant cytoplasm in this field (from Figure 1 photographed at high magnification).

FIG. 3. The pia arachnoid is involved by tumor as shown here.

FIG. 4. One of the cranial nerves is densely infiltrated by neoplastic cells.

always be implicated; particularly if its embryologic development is considered. The pineal and the ependyma are closely related in their development, and it may be that these tumors arise diffusely in the ependyma rather than always beginning as a tumor of the pineal and spreading to involve the ependyma. Histologically, the tumor in this patient fulfills all the morphologic criteria for a pinealoma which probably originated from both the ependyma and the pineal.

Final anatomic diagnoses: Primary: diffuse pinealoma involving the ependyma of the lateral, third and fourth ventricles and pineal gland; infiltration of cranial nerves, meninges and choroid plexus by pinealoma; cerebral edema with uncal grooving and focal necrosis of occipital lobes; atelectasis of lungs; pulmonary edema; bronchopneumonia, slight; congestion of viscera, moderate. Secondary: healed pyelonephritis; arteriosclerosis of aorta and coronary arteries, moderate; hypertrophy and dilatation of heart (420 gm.); focal fibrosis of myocardium; calcified nodules in lower lobe of right lung and in right peribronchial lymph node; obesity; diffuse nodular cirrhosis, slight.

Clinic on Psychosomatic Problems

Brief Psychotherapy of a Patient with Headache and Endometriosis

THESE cases are chosen to illustrate the relationship between psychiatric and medical factors in the production of symptoms. They are part of the Harvard teaching on the Psychiatry Service of the Massachusetts General Hospital. This psychiatric conference was edited by Dr. Erich Lindemann.

MR. LESTER H. GRANT (Harvard Medical School IV): This thirty-one year old occupational therapist was referred to us because of incapacitating headaches. The headaches had been frontal and bilateral, with associated nausea and vomiting and blurring of vision. They began in the morning and lasted at least two to three days. They were not improved by opiates, ergotamine, nicotinic acid, calcium lactate or barbiturates but were relieved when the patient lay down. The most recent attack had continued for over two weeks. The patient was referred by the hospital in which she worked because it was hoped that her health could be improved so that she could return to work.

At the age of fourteen the patient had had a ruptured appendix which had been treated by drainage, and seven months later removed. From a report from another hospital we learned that during the first year of her marriage, at the age of twenty-four, in November, 1947, she had had an operation for the removal of ovarian cysts. Two years later she had had a hysterectomy with bilateral salpingo-oophorectomy for endometriosis. At operation marked adhesive endometriosis of the entire pelvis was found. On the right side was a chocolate cyst, and on the left there was a smaller mass involving a tube and part of the ovary. In May, 1950, the patient was in the hospital for colicky pain in the right flank, with nausea and vomiting of five days' duration. A diagnosis was made of pyelonephritis and acute hydronephrosis on the right, with a stricture in the right lower ureter. The patient was readmitted in July, 1952, for a ureteral stricture with severe right lower quadrant pain, nausea and considerable flatus. It was decided that perhaps an endometrial implant in the right ureter was producing some constriction of the right ureter. The patient recovered after dilatation of the right ureter. In June, 1952, the patient was admitted for left lower quadrant pain. An intravenous pyelogram revealed a nonfunctioning left kidney and ureter. Accordingly a left retrograde pyelogram was taken, which showed no abnormality by x-ray. The patient was discharged with a diagnosis of left ureteral obstruction, etiology unknown; pelvic endometriosis; renal papillitis and mild cystitis.

The neurologic consultant at Massachusetts General Hospital learned that the patient first noted headache at the age of twenty, when her periods became irregular. The patient's husband reported to the other hospital in 1949 that the patient had headache. These headaches, however, were apparently less severe than the attacks which began in September, 1953, after radiation treatment. When she awoke she had a bilateral, supraorbital, throbbing headache, and vomited. The headache did not prevent her sleeping nor awaken her. She had dizziness, but no sensory or motor disturbances.

Shortly after admission to Massachusetts General Hospital she was seen by a medical consultant. His impression was of a severely disturbed female with a polysurgery syndrome who seemed to have attracted attention with her many symptoms. He found tenderness in the lower right quadrant and described her as looking ill, but not in acute distress.

For several days after admission the patient had urinary retention. The gynecologic consultant thought that distention of the bladder to 1,100 cc. was abnormal and that hysterectomy should not have caused parasympathetic nervous interruption to the bladder. He wondered if this did not represent an "hysterical bladder." He recommended catheterization and premarin,®

1.25 mg. three times a week for three weeks, omitting it for the fourth week.

The neurologist who saw her could not make a positive diagnosis. He found no features of disease which could produce cyclic headaches similar to those associated with abnormal menstrual flow. He found no evidence of otitis, sinusitis, herpes zoster, dental disease, abnormalities in the skull or cervical spine, or evidence of drug intoxication. He thought her history was not characteristic of migraine. He found her to be normotensive. She did not have histamine headache. He was reluctant to say it was hysteria without confirmatory evidence from the family history, marital and sexual history, symptoms preceding surgery, and job record.

Dr. Patricia Benedict, in consultation, thought that from an endocrine viewpoint the foremost problem was that of the menopausal state. Even though some ovarian tissue had been left behind at the time of the bilateral oophorectomy when the patient was twenty-six, this undoubtedly had ceased to function after x-radiation a year before. It was believed that estrogen therapy was indicated for the following reasons: (1) Certain metabolic effects of estrogen are especially desired in a young woman of thirty-one, e.g., prevention of atrophy of skin and premature aging, beneficial effect on bone structure; (2) approximation of a more normal hormonal pattern would leave her with one less stress to which she would have to adjust, this stress being a manifest physiologic abnormality which can be demonstrated by laboratory methods (cf. elevated values of follicle-stimulating hormone in the urine in menopausal state). The problem was to supply the advantageous features of estrogen therapy without, at the same time, reactivating the endometrial implants which probably remained and which had given trouble in the past. After presentation at endocrine rounds a program of therapy was outlined by Dr. Fuller Albright: sodium estrone sulphate (premarin), 1.25 mg. by mouth daily, plus methyl testosterone sublingually, 5 mg. daily, to be maintained indefinitely, and the patient to be followed carefully.

During the stay on the Psychiatry Service the patient had fifteen psychotherapeutic interviews, which she considered were designed to help her gain new insights and find better ways of handling her very real difficulties. On admission her behavior and stream of talk were normal. She was anxious, sad, depressed and vaguely apprehensive. She had good intelligence, good memory and some superficial insight into the fact that she had emotional problems.

The patient's father died of tuberculosis in a sanitarium when the patient was about seven. She does not remember having had much contact with him. When he came home on visit from the sanitarium he stayed in his room, and the children would go to his door "to say hello" to him. Once he took them to the store and bought them ice-cream cones. The family was brought up by the mother who was a strict parent. The patient had twin brothers a year older. One of them was her favorite, to whom she was close in childhood. This brother was killed during World War II. The other brother is described as irresponsible. He is unmarried and does not help support his mother.

The patient first spoke of her childhood as a time she enjoyed when she played with other children, and walked and played with her brother. She went on to say that it was "tough," too. Her mother was not well and could not do much work. She remembers how poor they were and how much they worried about money. Once a month they had to go to the Poor Farm to get food and clothes. These clothes came in a burlap sack; and when the children unpacked them, they knew they were all the clothes they would have for a while. They never fitted right and other children in school laughed at them.

The patient, however, did well in school and one year was voted the most popular member of her class. After high school she became an occupational therapist and worked in a large hospital. She was a hard worker and became the head of the department.

At the age of twenty-four she married a man she had known since the fourth grade. He was a Roman Catholic; she was Protestant. After marriage he went to graduate school where he became more and more absorbed in his studies. During this time she helped support him and made a great effort to be a good housekeeper. The marriage seemed to deteriorate after the first year. Her husband spent weekends studying and visiting his family, to whom the patient never felt close. He insisted that they drop her "ignorant" friends. She said that the only area of compatibility was in their sexual relations. She failed to become pregnant, although she wanted children. After her hysterectomy her husband "walked out on her" and said he would get a

divorce if he had to carry it to the Supreme Court. The patient herself got the divorce, but did not want it.

Psychotherapy was planned on a short term basis, aimed at restoring the patient's self-regard, and at the same time avoiding discussions of her symptoms. She had about fifteen interviews. In the fourth interview, after describing the deprivations of her childhood, the patient cried. She said she had wanted nice clothes all her life, and could get them only by making them. She wanted her health so that she could work. She wanted a husband but had lost him. After this outburst she apologized for crying and said that she had learned not to cry.

The next time she was seen, two days later, the interviewer opened a thermos of coffee and gave some to the patient, who said that it tasted better than hospital coffee. She said that her headache had disappeared the previous morning and had not returned. She talked about her feelings of bitterness, about how much she loved babies, and yet avoided seeing her friends' children. She wanted marriage but was afraid of being hurt by it again.

Later that day, after a discussion of having her hospital bill reduced, the patient started crying again. The interviewer apologized for reminding her of the Poor Farm and the second-hand bookstore. He went on to explain that she had difficulties with people she had trusted, and that she was now having favors done for her by strangers. This, he said, deprived her of a certain amount of independence. He pointed out that this was a dilemma in that while they forced dependence upon her, they at the same time wanted to help her to become independent and to acquire the best valuation of herself.

In subsequent interviews the patient discussed her lack of trust in people, her fear of offending someone and her fear of being hurt. She talked about her present boy friend, how she considered the relationship satisfactory as an outlet for her emotions, although she does not look forward to marrying him. She discussed her feelings of frustration, how music made her blue, how she could not stand Christmas Day and usually worked to avoid thinking about it. She expressed her inability to get what she wanted from life, said she might really want a man and might feel inferior to him, whereas she would like to stand on her feet and say she was as good as he was. After talking about other women who have babies and about how she avoids seeing her friends with babies, she began to cry. She was afraid she would not marry again, and that if she did her husband would not want to adopt children. She wanted to work with old people so that she would not hear talk about marriage and babies.

The patient brought up the possibility of her leaving the Hospital in the twelfth interview. She thought she felt better than she had in her whole life, certainly better than in the last few years. She wanted to see how she felt when she returned home. She also felt guilty about occupying a bed that someone else might need. The day after this discussion the patient complained of hot flashes and fatigue. The interviewer assured her that starting medication again would make her feel better, and pointed out that symptoms should not make it necessary for her to castigate herself over her anxiety, that she need not add more stress to what she had already. The possibility of her getting a job to work with children was discussed, and the patient thought she might enjoy that. Moving to Boston was considered and the patient wondered what her mother would say, whether she would refuse to come with her or whether she would simply tell her to leave if she wanted to.

In the final interview the patient expressed concern over possible return of poor health. She could never predict the onset of headaches, and usually felt well when they "hit her." She decided finally to take a job working with convalescent patients which would not be too taxing and where she would have less administrative work. She was discharged as improved, although she was somewhat apprehensive about the future and frightened that the headaches might return. There was, however, a strong realistic quality to her thoughts and determinations.

STAFF DISCUSSION

DR. ERICH LINDEMANN: This is a brief encounter with a very complicated problem, in which an inexperienced psychiatrist tried to achieve some change in a limited period of time. This type of short range therapy with limited goals has been more common than long term acquaintance with patients for more intensive therapy. The diagnosis is not completely settled.

DR. CARL BINGER: The fact that the headache was relieved by the patient lying down would not be diagnostic. Migraine headaches as well as those caused by tension are often relieved by patients lying down. The textbook descrip-

tion of migraine does not always conform to reality.

MR. GRANT: The neurologists did not think she had migraine. Also, the headache was not alleviated by trials of medication, including ergot.

DR. MORRIS CHAFETZ: The electroencephalogram showed 2 to 4 per second waves in the left temporal lobe. I should be interested to see what a sleep record would show, to see whether or not it would indicate a temporal lobe lesion.

DR. ELIZABETH R. ZETZEL: Did the headaches stop dramatically?

MR. GRANT: She has had no headaches since the first interview in which she cried. In the past she has had long periods of freedom from headache, however. They come on about every three months and have done so since the irradiation.

DR. GERALD CAPLAN: The fact that the headache disappeared on lying down seemed striking because one sees in the patient a tendency to lie down, to want to be a little girl again. She has a feeling of deprivation in relation to her mother. She believed that the only way to achieve success was to be a slave like her mother. However, in this life of hard work she feels deprived, and going to bed would fit in with this feeling. On a non-verbal plane you gave her a good mother-child relationship. You gave her things, got involved with her problem and gave her some feeling of independence. You acted out what I think is the central problem. It was interesting that you did not go into a discussion of her relationship with her mother.

MR. GRANT: She had a difficult time with her mother who was very restrictive. There were a lot of rules in that family, and talk about morals, ethics and dogma. I thought the girl had difficulty all her life freeing herself from her mother. When her marriage was breaking up, however, her mother said she had to stand on her own feet. The patient did seem to have an exaggerated sense of responsibility for her mother, who was somewhat domineering. I could not piece all this together into a clear picture of their relationship.

DR. LINDEMANN: The depressive component was impressive. Her life has been a sequence of losses of various kinds, including parts of her body. The threatening state of her pelvic organs, with the endometriosis, is remarkably underplayed. The excellent future she describes with her boyfriend seems like a denial. Is one part of the headaches displacement upwards of a grave concern?

DR. ZETZEL: I was struck by that. Whatever the nature of the headaches, they have an enormous significance to the patient. Mr. Grant was right in stressing the patient rather than her headaches in his treatment. He handled the transference well. In such brief, intense relationships one may find difficult and unmanageable transference situations. The way the patient was confronted by the dilemma between dependent needs and recognition that she did not want to be dependent was an important step. How far has this relationship therapy mitigated this patient's superego? Will she continue to be as self-punitive? The fact that she went to work in a convalescent home is a good sign.

DR. LINDEMANN: She went to the town in which her mother lives, however, and will continue looking after her. There seems to be an obligatory relationship between them.

MR. GRANT: If you tie her problem to the headache, you play a long shot. If you say that, regardless of the headache, her job is to find a way for her to help herself, whatever she gets is pure gain. The psychologic connection with the headache is really beside the point; for if you tie the problem to the headache and if you cannot relieve them, everything is lost. The problem, I thought, was to help the patient capitalize maximally on her many assets, regardless of the headaches.

DR. DANIEL C. DAWES: That is a good point. You have not made the mistake of treating the symptom and not the patient. What causes the headache is not so important.

DR. LINDEMANN: I should like to ask Dr. Hofmann to discuss this. He is concerned with the central European approach to problems of people along existentialist lines.

DR. HANS F. HOFMANN: You dealt with the patient and solved the headache in the context of the whole personality. The patient, for certain reasons, was unable to face reality. Her feeling of having to go home expressed that. It was excellent that you made it clear that the essential part in the therapeutic procedure is for the patient to become able to relate herself to a given situation with full realization of the implications. It is important to make it clear to patients that it is neither just the fact that they are well physically, or that the external situation changes, but that they are able to create their personality on the basis of their being human

beings in the conflicts of their situations. I think this patient's headaches disappeared when she could take a step toward realizing herself in her personal integrity. It is important not only to treat a patient as a person but also to help a patient see herself as a person. That we often forget in treatment. Only in achieving it can you have an active and creative relationship to the world surrounding the patient.

DR. LINDEMANN: This young woman had a number of events in her life which we consider tragic: she lost her father early, her favorite brother later and she had a strong sense of obligation which resulted in strong demands on herself for achievement. She seems to have, also, an inclination to make "fate go the wrong way." She was one of the best occupational therapists in a large hospital. That hospital asked us to help her so that she could return to work. She has achieved considerable status, but has a number of illnesses—endometriosis and headache. Her level of achievement seems to have been higher than her emotional resources, so headache or other illness enforces a pause in her work. The therapist has to decide whether to preserve this high level of performance for the hospital at the cost of occasional sickness, or whether to tackle the sickness and rearrange her life pattern more in keeping with her limited resources. With complete psychoanalysis one would have confidence in her using all her resources. With this more usual brief encounter the doctor has to decide where his obligations lie. He stands in a moral conflict of mutual obligations.

DR. HOFMANN: In that sense the headache and being in hospital would be necessary for the patient. In continental psychiatry there would be an inclination for personal necessities to prevail. In Zurich we would let the patient retreat until she improved. If you keep the patient confronted with the demands and obligations of society, she would not be able to restore and redevelop her resources. The first act in therapy would be to consider how far the personality structure was strong enough for the person to face creatively the given social situation. The second act would be to reinforce the given abilities so that a social adjustment can be made.

DR. LUCIE N. JESSNER: These two acts might have to go on together; the patient has the two tendencies so strongly herself that she might not be able to do it in two different acts. She wants to get what she has missed, a good father and mother; she wants a place where she can lie down. On the other hand, she has a strong feeling of obligation to do something. I would first minister to her dependent needs. She lives so much on denial and is so masochistic. Could one get her in a situation that would gratify her dependent needs, and also support her to follow her other line? The patient seems to be filled with longing. Music reminds her of the loss of her husband. She would like to have had a child, which is now out of the question. She has recurrent symptoms; I do not know why they recur every three months. She lives for awhile beyond her resources, and then is exhausted.

DR. MARIE LORENZ: Judging from the interviews that were read, there seemed to be a meagerness of personality and ability to talk about herself. If we catch a thread in the way she talks about herself, it comes from an undercurrent of regrets not about her present status; it is something concerned with her feelings—regrets for things she did not have as a little girl. Her problem is not so much taking a stand and asking where she is as a social being, as it is a simple need to have simple gratifications for her needs. I wondered how she felt when she cried. With a little opportunity to express emotions there was more expression of her own needs. She shows regret, wailing what she misses rather than a turning toward a more positive path of deciding which way she is heading. She would not put the problem that way herself; to her it would seem to be a problem of deprivation, loss, regret and anger, rather than going toward something she sees as a style or pattern of life.

DR. LINDEMANN: Dr. Lorenz mentions the patient as a social being; Dr. Hofmann says she has to recognize herself as a person. Doesn't that imply a significant cultural difference? A social being has a moral responsibility and responds to the demands of people around. As a person in Dr. Hofmann's sense, one may be proud of having achieved a significant mastery of a style of life, even though he is not seen as a very important person by others; he may also suffer from being below what he might expect to achieve. Curiously enough, in the work we do here that side of it is rarely verbalized, although it may be an important facet of a more integrated person.

DR. LEMOYNE WHITE: We had some definite ideas about this case. It was our impression that one of her real problems was that she fell so far below her own ideal. One key point in Mr.

Grant's approach was implicitly and explicitly to try to restore her self-regard. This was aided by Mr. Grant's own transference to this patient, in which this was not a therapeutic plan but an intuitive effort. This almost verged on anaclitic therapy. Mr. Grant remarks how much was given to this patient. The real turning point was when Mr. Grant gave her some coffee. That is why I used the term anaclitic. He actually performed this service of fulfilling the role of the good parent. Then he tried to express his confidence in her ability to perform. He kept away from symptoms because we thought that would stress her own badness. We reiterated that she was worth doing something for. Mr. Grant's many actions gave some substance to this statement. It certainly has had some elements of a reconstituting experience for this patient. How long it will last, I do not know.

Dr. Binger: We are much influenced by Dewey, James and others who thought in terms of the pragmatic viewpoints. That is a wholly different point of view toward the person.

Dr. Clemens E. Benda: American psychiatrists find it difficult to know what is going on in European psychiatry. Freud is written in German. His doctrine was influenced by the ideas of the nineteenth century. American psychiatrists, however, do not think of psychoanalysis in terms of its theoretic meaning, but rather of what can be done with it. Thus, from a practical standpoint the difference between existentialism and psychoanalysis is far less than would appear.

FOLLOW-UP

Mr. Grant: Just before this conference I telephoned the patient who told me she had been well since she left the hospital three weeks before, but that she was tired. She had made plans to get away from her regular job and to take a similar job in a different institution in her home town. She had also investigated the opportunities for training in dress designing and this was her goal —to switch to a field that had interested her all her life. The little girl who had to get her clothes at the Poor Farm seems finally to have reached a decision that most of all she would like to design dresses.

Dr. Patricia Benedict: So far (about two months) we have been lucky with our therapeutic program: she has no hot flashes and the endometriosis seems to be quiescent. She evidently found that she was happier in a hospital environment for she has returned to the place of her former employment, this time in a different capacity which she finds is more of a drain on her physical reserve than she cares to experience. In spite of the fatigue attendant upon her new duties she has had no headaches; she presents a neat, attractive appearance and would seem to be happy and well adjusted.

Mr. Grant: Two weeks after this it was learned that the patient made plans to move to another state, by herself, apparently in keeping with her resolution to carve out a new career. Two and a half months after the last seige of headaches, or about two weeks before a new attack might be due (if the previous pattern is to be followed), the patient was feeling fine and in good spirits.

SUMMARY

This case discussion is concerned with the limited goals which may be attained in psychotherapy in the setting of a psychiatric teaching service where rather young therapists get their first experience in handling the emotional and personality problems of medically sick patients. Although it was impossible to clarify completely the nature of this woman's headaches and even though in the short time available a complete study of her personality could not be made, it was felt that this woman's psychotherapeutic experience was of real benefit to her.

Case Reports

Diffuse Lymphosarcomatosis of the Central Nervous System Simulating Infectious Polyneuritis*

LT. COL. ROY E. CLAUSEN, JR., M.C., LT. COL. ARTHUR F. LINCOLN, M.C.
and CAPT. HENRY K. SILBERMAN, M.C.

Denver, Colorado

INVOLVEMENT of the nervous system by diffuse tumors is not new in the medical literature. In 1837 Ollivier[1] described a patient with "primary sarcoma" of the cerebellum with seeding of the tumor in the pia of the spinal cord. At autopsy the entire cord was enclosed in a crescent-shaped tumor. In 1870 Eberth[2] presented a subject with "epithelioma" of the meninges which he believed arose from the connective tissue of the subarachnoid space. He discussed several similar cases and concluded that primary diffuse tumors were rare when contrasted with those occurring secondarily from extraneural origin. In 1902 Nonne[3] reported a case of this type and reviewed the literature on the subject. His patient showed uniform microscopic infiltration of the pia without evidence of macroscopic tumor masses. He observed extensive perivascular infiltration of the Virchow-Robin space and theorized that the tumor may have arisen from the epithelium of the perivascular lymphatics. In the differential diagnosis, syphilitic meningitis or tuberculous meningitis were the most frequently considered anomalies. Because the clinical manifestations were frequently out of proportion to the destruction of nervous tissue, Nonne postulated that varying degrees of vascular compression or toxic factors played important roles in the production of the polymorphous clinical manifestations of these tumors. Rindfleisch[4] was one of the first to comment on the cerebro-spinal fluid findings in diffuse tumor of the central nervous system. He observed colorless cells in the fluid with peculiar nuclei, xanthochromia and pellicle formation.

Diffuse lymphosarcomatosis of the central nervous system is rare. In a study of 790 consecutive patients admitted to a large hospital with a presumptive diagnosis of poliomyelitis, Top and Brosius[5] found only one diffuse neoplasm of the spinal cord. Sparling et al.[6] reviewed 11,500 autopsies for direct involvement of the nervous system by malignant lymphomas. Of these, malignant lymphoma occurred in 118 cases. Twenty-seven of the 118 showed invasion of the central nervous system, and diffuse infiltration was seen in only five cases of leukemia. They concluded that lymphosarcoma caused fewer central nervous system manifestations than other lymphoid tumors. The clinical diagnosis, when made before death, usually followed the discovery of lymphosarcoma in other organs. In a case report by Kohut[7] the disease was not suspected, though the patient had received roentgen-ray treatment for systemic lymphosarcomatosis previous to the onset of the symptoms involving the central nervous system.

The clinical manifestations are variable. Schuberth[8] reported fifty-six patients with diffuse sarcomatosis and twenty patients with diffuse gliomatosis in the literature to 1926. A summary of his clinical findings is presented in Table 1. The spinal fluid was examined in only one-third of the cases reported by Schuberth. Most showed a mild lymphocytosis; in ten cases tumor cells were found. Elevated pressure and protein were noted in several instances, and

* From the Neurology Service, Department of Neuropsychiatry and the Research and Development Branch, Fitzsimons Army Hospital, Denver 8, Colo.

xanthochromia was noted in a few. Fried[9] reviewed thirty cases of leukemic involvement of the central nervous system. Among these were seven showing cranial nerve impairment and eleven showing affection of the spinal cord and nerve roots. Only three of the eleven showed

TABLE I
FREQUENCY OF CLINICAL MANIFESTATIONS*

Clinical Manifestations	Per cent
Presenting complaints	
Headaches	87
Vomiting	45
Cranial nerve disturbances	84
Optic nerve	63
Papilledema	59
N-III, IV, VI	42 (VI: 32)
N-VII	34
Spinal cord manifestations	
Motor signs	70
Flaccid paralysis	32
Sensory diminution	53
Root pain	39
Nuchal rigidity	29

* Data in fifty-six cases of diffuse sarcomatosis and twenty cases of diffuse gliomatosis according to Schuberth.[8]

direct invasion of the spinal cord by leukemic cells. Garvey and Lawrence[10] presented two examples of facial diplegia occurring in the course of lymphatic leukemia. Tromner and Wohlwill,[11] reviewing the nervous complications of leukemia, noted that the most frequently affected cranial nerves were the facial and the acoustic nerves. Schwab and Weiss[12] reported the nervous complications of leukemia occurring in patients at the Boston City, Huntington Memorial and Massachusetts General Hospitals in a twenty-year period In those cases 30 per cent showed anesthesias or cranial nerve palsies, and only 7 per cent showed meningeal signs.

In diffuse tumors of the central nervous system, spinal fluid pleocytosis is common and may be variable, often rising to high levels. In a case reported by Schwab and Weiss,[12] the count ranged from 280 to 10,000 cells per ml. Similarly high cell counts have been described by Nedelmann[13] and by Murphy and Brody.[14]

Many have described the presence of malignant cells in the cerebrospinal fluid and have commented on their diagnostic importance.[14–18] Baumann[19] commented that the disease was difficult to discover during life unless the tumor cells were found in the cerebrospinal fluid. Brown and Kernohan[20] stated that they had never seen a case of diffuse tumor of the central nervous system in which the diagnosis had been made sufficiently prior to death to institute appropriate therapy. The diagnosis depends on the demonstration of the tumor cells in the stained spinal fluid sediment, and rapid centrifuging is discouraged lest cell morphology be altered. Hassin and Bassoe[21] suggested that in case of a dry tap the needle tip be examined for the presence of cells upon withdrawal. High protein levels, pellicle formation and xanthochromia are relatively common.[4,8,12,15] Cerebrospinal fluid pressure may be increased due to cerebral involvement or may be reduced due to block of the subarachnoid space by tumor. Berg[22] has recently reviewed the literature relating to all types of diffuse central nervous system tumors, with special reference to the spinal fluid sugar levels, and finds that it is reduced in 75 per cent of the cases, sometimes to very low levels or total absence. Though we are primarily interested in the lymphoid tumors, Berg's study showed that twenty of fifty-seven patients reviewed by him had carcinoma. It is apparent that the principles discussed in this review may apply to any type of diffuse tumor of the nervous system.

CASE REPORT

A seven year old white male child was admitted to an Army Station Hospital on March 17, 1953, with pain in the left elbow of three weeks' duration and pain in the left leg, about the knee, of one week's duration.

Three weeks prior to this admission, the patient became aware of pain in the left elbow which radiated proximally and distally. There was local tenderness but no swelling. Two weeks before entering the hospital he noticed pain in his left leg about the knee with associated tenderness. About March 6th he developed headache and photophobia. On March 11th he was unable to turn his left eye to the left side upon conjugate deviation. He kept his head turned to the right. He was seen by a civilian physician on March 16th who noted blurring of the margins of the optic discs. Deep tendon reflexes were absent in the lower extremities and those in the upper extremities were hypoactive. The abdominal skin reflex was absent in the right upper quadrant. The Kernig and Brudzinski signs gave

positive results. There was a pruritic papular rash on the right side of the trunk, questionable weakness of the left upper extremity and limited dorsiflexion of the left foot. Lumbar puncture showed an initial pressure of 170 mm. of water and a final pressure of 130 mm. No cells were seen. The total protein level was 18 mg. per cent; sugar was 76 mg. per cent. Roentgenograms of the skull showed questionable erosion of the posterior clinoid processes and of the right lesser sphenoid wing. He was referred on March 17th to an Army Station Hospital with the presumptive diagnosis of intracranial neoplasm or disease. At this installation the patient was noted to be afebrile, and essentially the same physical findings were noted as those previously described. In addition, he walked with a limp on the left side and displayed unequivocal weakness of the extremities on the left. The finger-to-nose test revealed bilateral ataxia. There was evidence of ataxia on performing the heel-to-knee test with the left leg. An electroencephalogram showed a diffuse, nonspecific slow wave abnormality without localization. The presumptive diagnosis was intracranial neoplasm, and the patient was transferred to an Army General Hospital for further observation and treatment on March 19th.

Past history and family history were not contributory to the present illness.

Physical examination revealed the following: Temperature 98.2°F.; pulse, 100; respiration 24; blood pressure, 110/70, and weight, 58 pounds. A papular erythematous rash on the right side of the body was noted. A purulent exudate was present on the left tonsil. The remainder of the general physical examination was within normal limits. Neurologic examination showed a stiff neck; positive Kernig and Brudzinski signs were present. The left pupil was larger than the right but both reacted well to light and accommodation. There was venous engorgement in the right fundus, and slight papilledema of the left optic disc. There was paresis of the left lateral rectus muscle and of the right internal rectus muscle with ptosis of the right upper lid.

The remaining cranial nerves were unimpaired. There was weakness of the paravertebral musculature of the back, the dorsiflexors of the feet, the left hamstrings and the left gluteus muscles. The deep tendon reflexes were absent throughout, as were the right abdominal reflexes. Babinski's sign was found on the right. No ataxia or sensory change could be elicited.

Laboratory findings revealed the following: Initial blood studies showed a red blood count of 3.6 million and a white blood count of 11,000; the differential count was within normal limits. The blood picture did not vary significantly during the patient's hospitalization. At no time were abnormal lymphocytes found in the peripheral blood. Proteus OX2 and OX19 tests gave negative results. Complement fixation tests for lymphocytic choriomeningitis and herpes simplex gave negative results. Urine examinations for coproporphyrins and lead also gave negative results. Spinal fluid observations were significant and are listed in Table II.

TABLE II
SPINAL FLUID FINDINGS IN A CASE OF DIFFUSE LYMPHOSARCOMATOSIS

Date	Cell Count (mm.³)	Differential		Total Protein (mg. %)	Sugar (mg. %)
		Lymphs. (%)	Polys. (%)		
March 17	0.0	...	...	18	76
March 19	4.0	100	0.0	56	84
April 13	64.0	92	8	210	71
April 24	217.0	95	5	485	63
May 4	597.0	98	2	...	..
May 17*	512.0	100	0.0	460	41

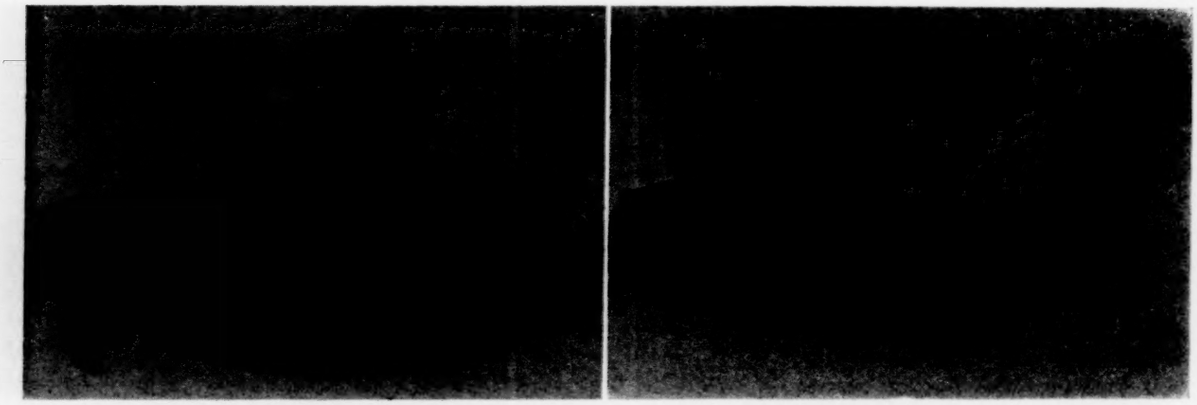
* Atypical cells suggestive of malignancy are present (A.F.L.).

X-rays of the skull on March 20th and June 10th revealed no evidence of intracranial disease. A long-bone survey on May 6th gave negative results. Films of the chest were negative A fluoroscopy on May 29th showed that the diaphragm failed to move with respiratory effort.

On March 20th, the day following the patient's admission, the paralysis had advanced to include all muscles of the left hand and the extensors of the left wrist. Although the patient remained afebrile, the most likely clinical diagnosis was believed to be poliomyelitis. The patient was drowsy but not disoriented or delirious. On March 22nd the patient developed weakness of the lower left portion of the face and had some trouble with swallowing and breathing. His condition was relatively static until the middle of April at which time the facial weakness increased to include both sides of the face. There was a facial diplegia of the peripheral type on April 13th. The left pupil was larger than the

right. The right optic nerve head appeared normal but the left blurred. The deep tendon reflexes were hypoactive on the right and absent on the left. Babinski's sign was elicited on the right. There was a flaccid paralysis of all muscle groups of the left lower extremity and weakness of the muscles of the shoulder girdle bilaterally, the left triceps, the left extensors of the wrist, the right quadriceps and the dorsiflexors of the right foot. At this time the cerebrospinal fluid (Table 1) showed an increase of cells and a marked increase of total protein. This, with facial diplegia, the patient remaining afebrile, and the subacute course led to the diagnosis of infectious polyneuritis. He was then given BAL but the condition failed to improve. On May 12th he was re-evaluated neurologically. There was an increase in the spinal fluid cell count and the total protein level with pellicle formation. He had bilateral papilledema which was more marked on the left side. The facial diplegia was less severe. The reflexes and weakness of the extremities were the same as previously noted but meningismus had increased to the point that slight extension of the extremities produced agonizing pain. A diffuse neoplasm of the central nervous system was considered and a cytologic examination of the spinal fluid for malignant cells was performed. A single group of cells, atypical and thought to be malignant, was reported but the results of this examination were thought to have given insufficiently conclusive evidence so the diagnosis of infectious polyneuritis was retained. On May 17th ACTH therapy was instituted. The child was placed in a chest respirator because of increasing weakness of the respiratory muscles. There was never a clinical response to ACTH of sufficient degree to warrant complete removal from the respirator, though by June 24th a partial removal was instituted. This patient never showed evidence of sensory impairment until the last two weeks of his illness at which time some ill-defined sensory loss with inconstant margins on the trunk and extremities was observed. On June 26, 1953, the patient died following a sudden cessation of respiration which failed to respond to resuscitative measures.

Postmortem Examination. The body was that of an eight year old white boy with marked atrophy and emaciation of the muscles of the legs and arms. No superficial lymph nodes could be palpated. The omentum was diffusely infiltrated by firm, white tumor tissue. The mesenteric lymph nodes were enlarged, firm and upon section had a glistening "fish flesh" appearance. The diaphragm was diffusely infiltrated by firm, white tumor. The spleen was not enlarged nor was it unusual. The wall of the gallbladder was infiltrated by tumor but the biliary ducts were patent. The pancreas was diffusely enlarged as were numerous pancreatic lymph nodes. All were matted together by tumor. Pancreatic ducts could not be visualized and multiple sections through the pancreas showed almost complete replacement by the tumor tissue. There was a small tumor nodule in the cortex of the left adrenal gland. On the greater curvature of the stomach toward the anterior wall there were giant rugae which were elevated 3.5 cm. above the uninvolved mucosa. (Fig. 1.) The entire area measured 4.5 cm. There was no ulceration of the mucosa. In the jejunum, about the middle third portion was an annular lesion which had not ulcerated the mucosa or the serosa but had involved the entire wall of the bowel. (Fig. 2.) It was grayish white and firm and had decreased the lumen of the jejunum by two-thirds. The lesion had raised, rolled edges and an umbilicated center. It gave the appearance of a "horseshoe" wrapped about the gut. Thirty cm. distal from the first lesion in the upper ileum was another annular lesion grossly similar to the lesion in the jejunum. Twenty cm. proximal to the ileocecal valve was another annular lesion which did not ulcerate the mucosa and was white and firm. The lumen of the bowel was patent, though decreased. Numerous small, white, subcapsular nodules were found in the right kidney, and in the left kidney there were three 0.3 by 0.4 cm. tumor nodules noted in the cortex and medulla. Upon opening the dura the cerebrospinal fluid gushed out under increased pressure. The brain weighed 1,323 gm. and was enlarged. It was soft and mushy. There was a deep pressure cone about the base of the cerebellum. The meninges were opalescent and grayish. Upon sectioning, the brain did not appear grossly unusual. The meninges of the spinal cord were thickened and opalescent. The ventral roots and posterior root ganglia were all enlarged, firm and, upon section, appeared to be grossly involved by the tumor. Some of the larger anterior roots and posterior root ganglia measured 1.0 by 0.5 cm. The remainder of the organs were essentially normal when observed grossly. The tissue was



1 2

FIG. 1. Gross appearance of stomach. Note the giant rugae without ulceration of the mucosa.

FIG. 2. Gross appearance of jejunum showing the annular lesion completely surrounding the gut.

fixed in 10 per cent formalin and sections were stained with hematoxylin and eosin.

The immediate cause of death as determined by autopsy was diffuse lymphosarcomatosis.

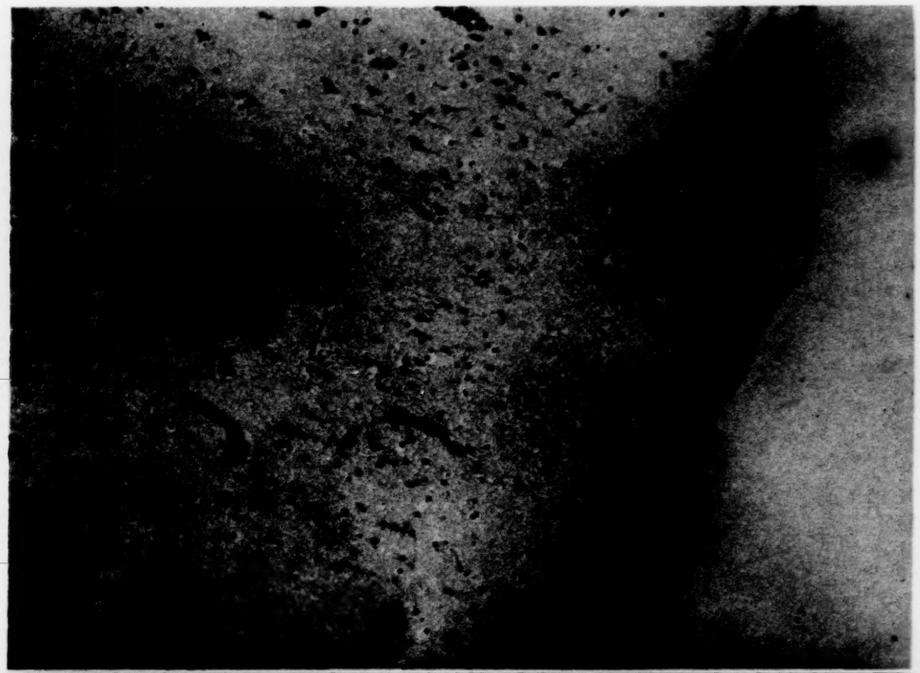
Histopathologic Examination. The peripheral lymph nodes taken from the cervical, inguinal and axillary chains were microscopically normal. The abdominal lymph nodes, however, showed complete distortion of their structure and were composed of unvarying sheets of atypical lymphocytes which had filled the medullary spaces and infiltered through and beyond the capsules of the lymph nodes. The nuclei of these cells were large and hyperchromic and the cytoplasm was scanty. Many mitotic figures per high power field were noted throughout. At the base of the lower lobe of the left lung there was diffuse infiltration of the parenchyma by lymphosarcoma cells. These were also found in the subpleural lymphatics of both lungs. The heart was diffusely infiltrated by lymphosarcoma cells.

The parenchyma of the spleen, liver and adrenals all had lymphosarcoma cells throughout. The pancreas was heavily infiltrated with malignant lymphocytes and its structure had been almost destroyed. There was obstruction and dilatation of the pancreatic ducts. The islets of Langerhans were isolated in sheets of lymphosarcoma cells.

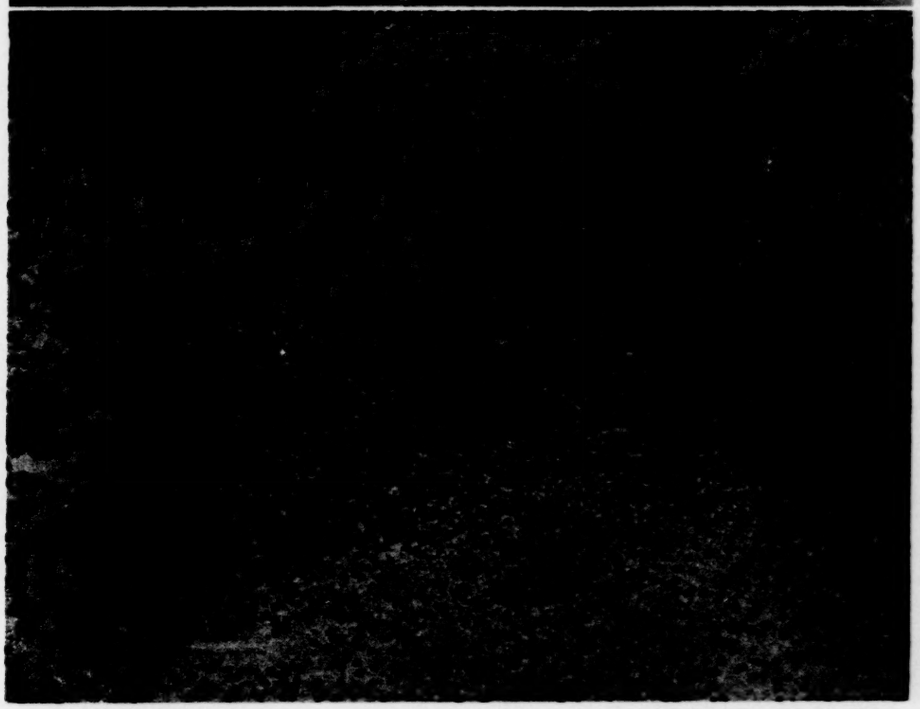
Sections through the giant rugae found in the stomach showed no evidence of ulceration; however, the gastric mucosa was diffusely infiltrated with sheets of lymphosarcoma cells which extended into and obliterated the submucosa. There were occasional clusters of lymphosarcoma cells beneath the serosa. There was no evidence of infiltration by the tumor between the muscular coats. Sections through the annular lesions found in the jejunum and ileum revealed no mucosal ulceration but diffuse and complete infiltration of the entire wall of the ileum and jejunum by sheets of lymphosarcoma cells was noted. Focal areas of lymphosarcoma cells were present in the medulla and cortex of the kidneys. There was also diffuse kidney subcapsular infiltration bilaterally.

There was diffuse involvement of the meninges by malignant cells which had extended into the Virchow-Robin spaces. The cerebral vessels and spinal cord vessels showed marked cuffing by the lymphosarcoma cells. (Fig. 3.) Beneath the ependyma of the ventricles there was heavy, diffuse infiltration by lymphosarcoma cells. This extended into surrounding brain tissues. The deep cortex showed similar diffuse involvement by malignant lymphocytes. (Fig. 4.) The mid-brain, pons, medulla, cerebellum and spinal cord showed diffuse infiltration by the tumor. Ventral roots and posterior root ganglia were replaced by unvarying sheets of lymphosarcoma cells. In some ganglia only an occasional ganglion cell could be found. (Figs. 5 and 6.) The peripheral nerves studied were not involved by the tumor.

Multiple bone marrow smears and sections of bone marrow taken from the sternum, ribs and vertebrae appeared normal. There was no evidence of involvement of the bone marrow by the malignant lymphocytes.



3



4

FIG. 3. Photomicrograph of cerebral cortex revealing diffuse cellular infiltration of meninges and vascular cuffing; × 200.

FIG. 4. Photomicrograph of cerebral cortex. Note the diffuse, deep infiltration by lymphosarcoma cells; × 300.

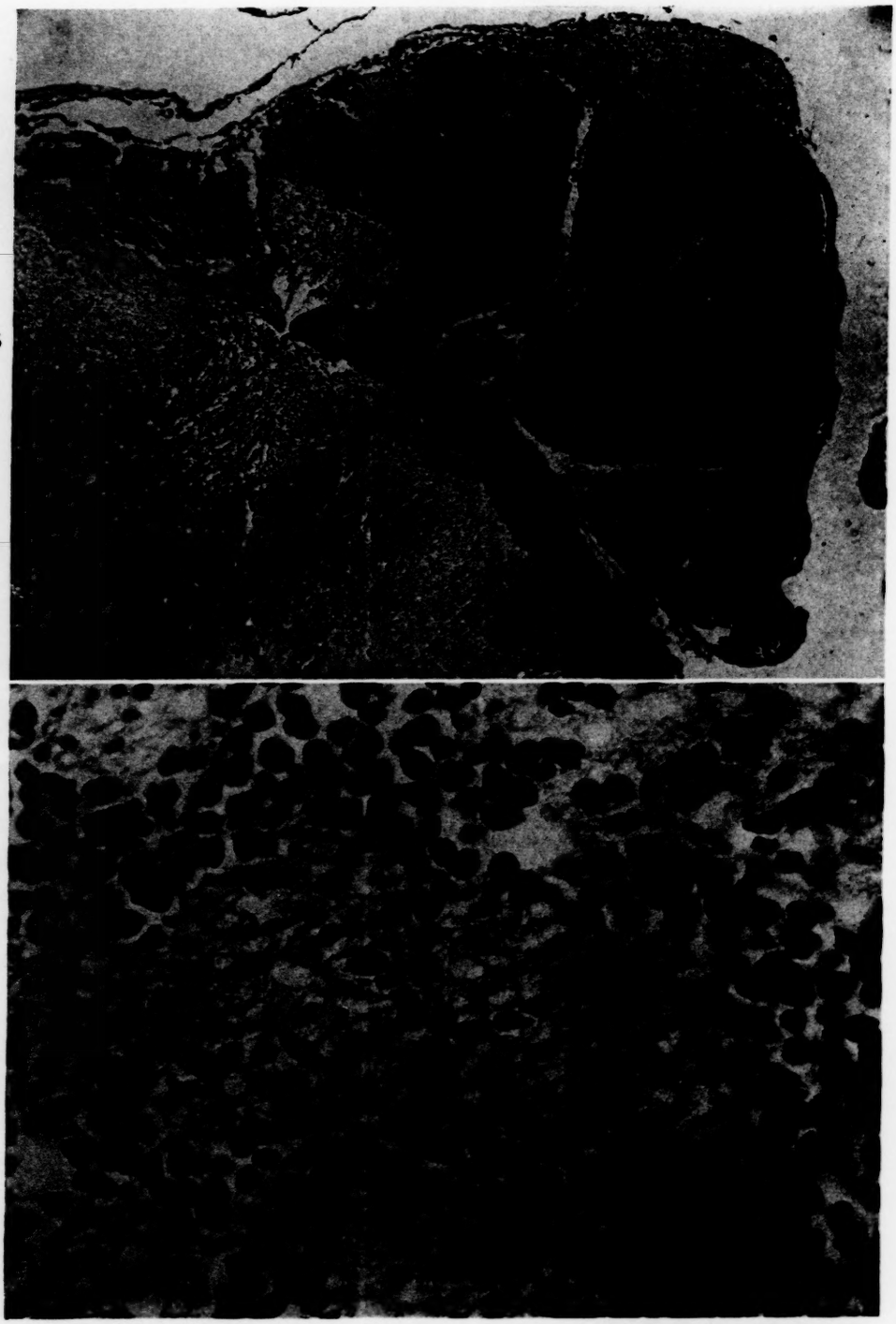


FIG. 5. Photomicrograph of spinal posterior root ganglion. There is extensive involvement by tumor cells; × 100.

FIG. 6. Photomicrograph of spinal posterior root ganglion at higher power; × 1500.

COMMENTS

Serebrjanik[23] believed that most of the diffuse lymphosarcomas invading the central nervous system begin with intra-abdominal primaries and subsequently spread into the neuraxis by perineural or perivascular transmission, with parenchymatous infiltration occurring as a later phenomenon.

Neural destruction may not always be parallel with the severity of clinical observations. As a result there have been attempts to explain the neurologic impairment on the basis of a secondary meningoencephalitis due to a reaction to tumor cells.[18] Others have postulated toxic products of the tumor cells as a cause of neurologic impairment. Alajouanine et al.[24,25] considered this a possibility in explaining the clinical signs in diffuse metastases in a case of pinealoblastoma and another of lymphocytic hyperplasia in which the severity of the findings could not be explained on the basis of the amount of nervous tissue destroyed. Schewtschenko[26] offered several theories concerning mechanisms which might protect the central nervous system against invasion by metastasis: (1) the parenchyma was protected against metastatic invasion by the epithelium of the choroid plexus; (2) the meninges provide protection against invasion; (3) this protection may be due in part to histiocytes; (4) the blood-brain barrier may play a part and (5) the arachnoid space, by means of its trabeculated construction, provides further protection. Extensive neural destruction in the absence of appropriately severe clinical findings is rarely seen, although Griepentrog[27] described a patient with diffuse meningeal sarcomatosis with extensive invasion of the spinal cord in which the neurologic picture was not as severe as might have been expected from the pathologic picture.

In the case reported, Schewtschenko's[26] theories are not well supported by the clinical and pathologic studies. The early appearance of cerebral signs occurring concomitantly with the peripheral manifestations suggests that the cerebral lesions probably started about the same time. Parenchymatous involvement was even well advanced deep in the cerebrum in the periventricular nervous tissue. The pathologic examination showed that in this case the nervous tissue involvement was adequate to explain the clinical signs, with the exception of the singular lack of sensory manifestations in the presence of heavy infiltration of the dorsal root ganglia. The extensive development of this disease in the abdominal viscera supports Serebrjanik's[23] observation that most primaries begin intra-abdominally.

Certain points are important for early diagnosis. Discovery of the malignant cells in the stained sediment of the cerebrospinal fluid determines diagnosis. A specific attempt must be made to discover atypical cells when a pleocytosis exists which cannot be adequately explained on the basis of infection. Chamber count differentials are not adequate to identify malignant cells. The spinal fluid should be centrifuged slowly, as recommended by Brown and Kernohan,[20] and the sediment then smeared and stained for cytologic examination. Studies of smears made from the puncture needle tip in the event of a dry tap is suggested by Hassin and Bassoe.[21]

An afebrile subacute course with progressive paralysis of the lower motor neurone type involving either spinal or cranial nerves and associated with increasing cerobrospinal fluid cell count and protein levels, with or without pellicle formation and spontaneous coagulation, should suggest diffuse tumor. As indicated by Berg,[22] low spinal fluid sugar levels should also arouse suspicion of diffuse tumor. In this case, however, the spinal fluid sugar was never at critically low levels, although just before death it was 42 mg. per cent. Disproportion between sensory and motor observations does not preclude the diagnosis.

SUMMARY

1. A brief review of the literature concerning the general aspects of diffuse tumor of the central nervous system, emphasizing principally those of lymphoid origin, is presented.

2. A case of diffuse lymphosarcomatosis involving the central nervous system with silent visceral involvement is reported.

3. The clinical and pathologic features of the case are discussed in relation to prevailing theories as to the nature of the nervous manifestations of these tumors.

4. Criteria for early diagnosis of diffuse malignancy of the central nervous system are presented.

REFERENCES

1. Ollivier, C. P. On the Spinal Cord and Its Diseases, 3rd ed.[3] 1837.

2. Eberth, C. J. Origin of epithelioma of the pia and of the lung. *Virchows Arch. f. path. Anat.*, 49: 1870.
3. Nonne, M. Diffuse sarcomatosis of the pia of the entire central nervous system. *Deutsche Ztschr. f. Nervenh.*, 21: 396, 1902.
4. Rindfleisch, G. E. Diffuse sarcomatosis of the spinal and cerebral leptomeninges, with characteristic changes in the cerebrospinal fluid. *Deutsche Ztschr. f. Nervenh.*, 26: 135, 1904.
5. Top, F. H. and Brosius, W. L. Diffuse sarcomatosis of the meninges suspected to be poliomyelitis; differential diagnosis of a case. *J. Pediat.*, 10: 27, 1937.
6. Sparling, H. J., Adams, R. D. and Parker, F. Involvement of the nervous system by malignant lymphomata. *Medicine*, 26: 285, 1947.
7. Kohut, H. Unusual involvement of the nervous system in generalized lymphoblastoma. *J. Nerv. & Ment. Dis.*, 103: 9, 1946.
8. Schuberth, O. Diffuse sarcomatosis and gliomatosis in the meninges. *Deutsche Ztschr. f. Nervenh.*, 93: 34, 1926.
9. Fried, B. M. Leukemia and the central nervous system, with a review of 30 cases from the literature. *Arch. Path. & Lab. Med.*, 2: 23, 1926.
10. Garvey, P. H. and Lawrence, J. S. Facial diplegia in leukemia. *J. A. M. A.*, 101: 1941, 1933.
11. Tromner, E. and Wohlwill, F. Involvement of the spinal nerves in leukemic disease of the nervous system. *Deutsche Ztschr. f. Nervenh.*, 100: 233, 1927.
12. Schwab, R. S. and Weiss, S. Neurologic aspects of leukemia. *Am. J. M. Sc.*, 189: 766, 1935.
13. Nedelmann, E. Malignant tumor of the thymus metastasizing to the nervous system by way of the spinal fluid. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 115: 539, 1928.
14. Murphy, J. P. and Brody, B. S. Nerve root infiltration in myeloid leukemia. *J. A. M. A.*, 115: 1544, 1940.
15. Critchley, M. and Greenfield, J. G. Spinal symptoms in leukemia. *Brain*, 53: 11, 1930.
16. Misch, W. Diffuse blastomatosis of the spinal cord with clinical symptoms of meningitis. *Deutsche Ztschr. f. Nervenh.*, 111: 233, 1929.
17. Marchand, L. and Picard, J. Diffuse primary meningeal sarcomatosis. *Ann. d'anat. path.*, 6: 1122, 1929.
18. Korbsch, H. The so-called meningitis tumorosa—pathological and clinical picture. *Nervenarzt.*, 5: 67, 1932.
19. Baumann, C. So-called tumorous meningitis. *Monatschr. f. Psychiat. u. Neurol.*, 86: 217, 1933.
20. Brown, M. H. and Kernohan, J. W. Diffuse meningiomatosis. *Arch. Path.*, 32: 651, 1941.
21. Hassin, G. B. and Bassoe, P. Disseminated sarcomatosis (melanoblastoma?) of the central nervous system and meninges; case. *Arch. Neurol. & Psychiat.*, 21: 1087, 1929.
22. Berg, L. Hypoglycorrhachia of non-infectious origin: diffuse meningeal neoplasia. *Neurology*, 3: 811, 1953.
23. Serebrjanik, B. Lymphogranulomatous meningoencephalitis and polyradiculitis. *Deutsche Ztschr. f. Nervenh.*, 129: 103, 1933.
24. Alajouanine, T., Bertrand, I., Castaigne, P. and Blatrix, C. Pinealoblastoma with extensive meaningeal and radicular involvement. *Rev. neurol.*, 83: 268, 1950.
25. Alajouanine, T., Castaigne, P., Lhermitte, F. and Graveleau, J. Sensorimotor polyneuritis in a patient with lymphohistiocytic hyperplasia. *Rev. neurol.*, 83: 294, 1950.
26. Schewtschenko, J. G. Defense reaction of zone of passive and active immunity to diffuse sarcomatosis. *Arch. f. Psychiat.*, 90: 556, 1930.
27. Griepentrog, F. Diffuse meningeal sarcomatosis. *Arch. f. Psychiat.*, 188: 549, 1952.

Symmetric Peripheral Gangrene Complicating Acute Myocardial Infarction*

ROBERT T. COTTON, M.D. *and* D. R. BEDFORD, M.D.

Topeka, Kansas

GANGRENE of symmetric distribution occurring in association with prolonged vascular collapse and independent of vascular occlusion has been infrequently observed. This report concerns such a case.

CASE REPORT

A sixty-four year old white salesman had had hypertension for eight years and substernal tightness on exertion for two years.

On the afternoon of October 5, 1952, while working on his car, severe, crushing, aching, upper substernal pain developed which radiated into both arms. He continued to work despite progressively severe pain accompanied by extreme weakness and profuse perspiration. When first seen at his home one-half hour later he was in profound shock with marked pallor, cold clammy skin, a weak slow pulse (rate, 50 per minute) and a blood pressure of 85/60. Following administration of morphine he was taken by ambulance to a local hospital; oxygen was administered enroute.

Pertinent physical findings were limited to the heart and these included poor heart tones and Grade II blowing systolic murmur. The lung fields were clear, the liver was not palpable and no peripheral edema was noted. An electrocardiogram (Fig. 1A) revealed an acute posterior myocardial infarction with a 2:1 A-V block and delayed A-V conduction (P-R interval .26). The blood showed 5.1 million erythrocytes per cu. mm., 15.5 gm. per cent of hemoglobin and 23,400 leukocytes per cu. mm., with 79 per cent neutrophils including 3 stab forms and 2 metamyelocytes.

Treatment consisted of bed rest, oxygen by nasal catheter, morphine, papaverine and dicumarol. The anticoagulant therapy was discontinued on the tenth day of hospitalization due to unusual dicumarol sensitivity resulting in hematuria. Pressor drugs were not employed.

Blood pressure was 122/82 one hour after admission to the hospital. During the initial forty-eight hours of hospitalization it ranged from 90/68 to 128/78 and the pulse pressure varied from 16 to 64 mm. Hg (average 38 mm.). Over one nine-hour period during the first twenty-four hours, the systolic pressure did not exceed 100 mm. Hg and the pulse pressure averaged 22 mm. Hg. Normotension prevailed after the first forty-eight hours. The pulse rate remained at about 50 per minute until the third day of hospitalization when it fell to between 30 and 40 per minute. An electrocardiogram (Fig. 1B) on that day showed a 3:1 A-V block alternating with complete A-V dissociation. During the fifth and sixth days of hospitalization the pulse rate rose gradually to 110 per minute and then became stabilized at between 100 and 110. An electrocardiogram (Fig. 1C) on the seventh day of hospitalization revealed restoration of sinoauricular rhythm and a normal P-R interval.

The patient's early hospital course was stormy; he experienced nausea and vomiting, recurrent chest pain and periods of restlessness and disorientation. Urine output was scanty, and the serum non-protein nitrogen on the fifth day of hospitalization was 141 mg. per cent. A fatal termination was expected.

On the third day of hospitalization the patient complained of sharp pains in both feet but presented no objective findings in the extremities. On the fourth day of hospitalization the lower extremities were noted to be cyanotic, and on the fifth day they exhibited definite purplish discoloration. By the sixth day the distal halves of both feet were black and cold with a sharp line of demarcation between the involved and

* From the Medical Service, Winter Veterans Administration Hospital, Topeka, Kansas. Presented at the regional meeting of the American College of Physicians, March 19, 1954, Topeka, Kansas.

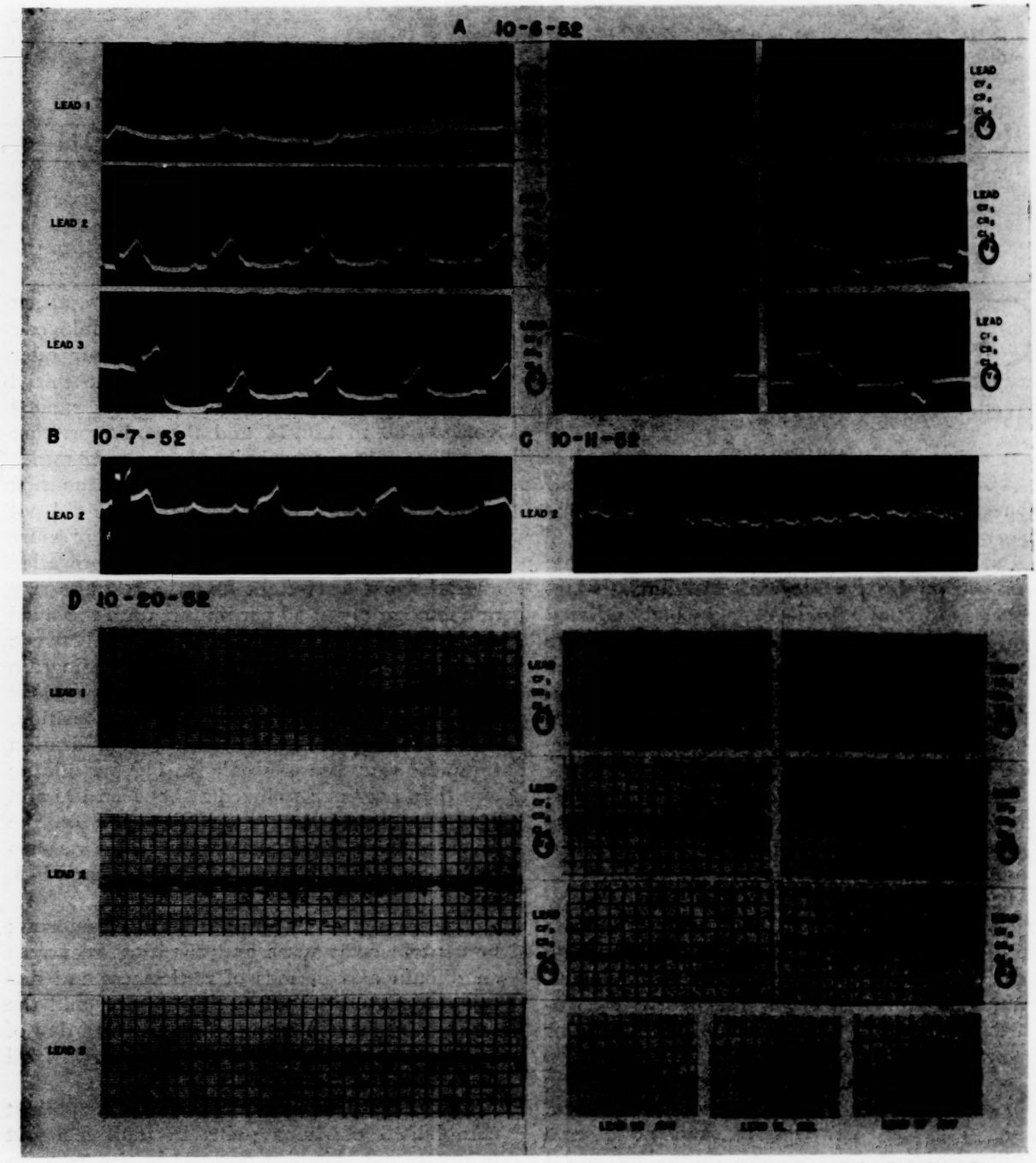
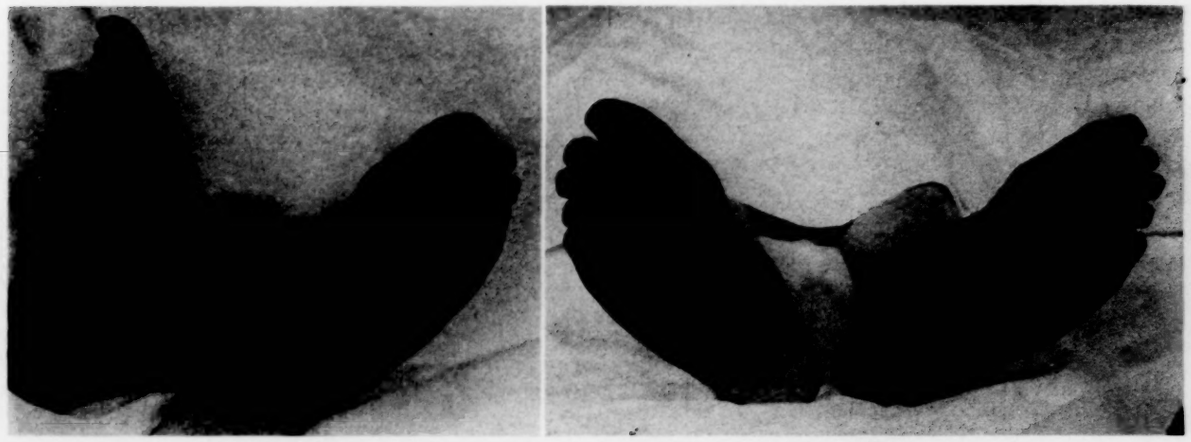


FIG. 1. Serial electrocardiograms showing fresh posterior myocardial infarction and progression of rhythm disturbances.

uninvolved skin. Despite evident symmetric gangrene, femoral, popliteal, dorsal pedal and posterior tibial pulsations were present bilaterally. By the following day discoloration had extended upward to the ankles, and blisters had appeared on both feet. During the next several days, however, the discoloration receded slightly.

Gradually the patient's general condition improved and on October 20, 1952, he was transferred to the Winter Veterans Administration Hospital, Topeka, Kansas, for continued care. Physical examination upon admission revealed Grade II arteriosclerotic retinopathy, distant heart sounds and sharply demarcated dry gan-



2A 2B

FIG. 2. Gangrene of feet. A, dorsal surface; B, plantar surface.

grene involving both feet. (Fig. 2.) The skin of the toes and soles of both feet was black, dry and of wooden consistency. The distal third of the dorsal surface of the right foot was dark purple. Blebs containing bloody serum were present about the edges of the gangrenous areas. The skin immediately proximal to the gangrenous area revealed normal color and temperature. Bilaterally the femoral and popliteal pulses were normal. The dorsal pedal pulsations were slightly less forceful. The left posterior tibial pulsation was easily palpable, but the right was absent. Blood pressure was 122/82, and the pulse was regular at a rate of 88 per minute. The blood showed 4,930,000 erythrocytes per cu. mm., 14.1 gm. per cent of hemoglobin and 12,100 leukocytes per cu. mm. with 41 per cent neutrophils, 55 per cent lymphocytes, 2 per cent monocytes, 1 per cent eosinophils and 1 per cent basophils. Sedimentation rate was 21 mm. per hour. Blood urea nitrogen was 10 mg. per cent, glucose was 110 mg. per cent and serum cholesterol 158 mg. per cent, all fasting values. An electrocardiogram showed sinus rhythm and findings typical of a recent posterior myocardial infarction. (Fig. 1D.) The patient was maintained at bed rest; he offered no complaints.

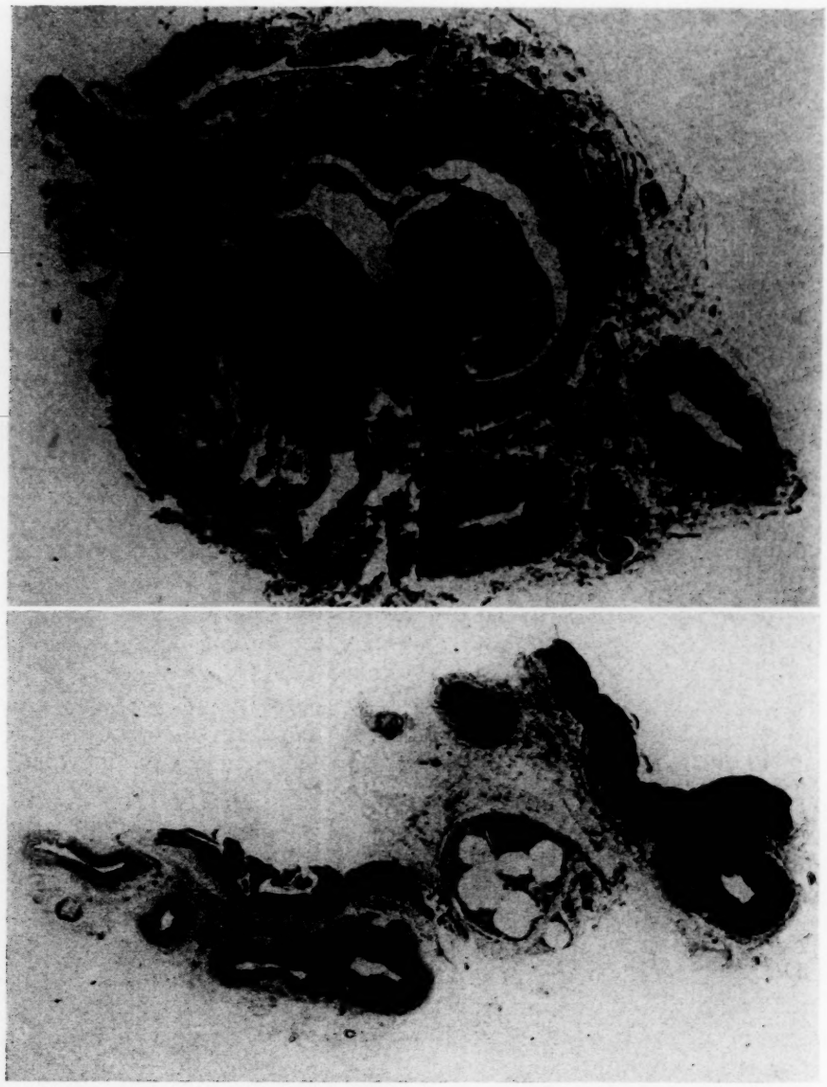
The gangrene of the dorsal surface of the right foot was superficial with progressive desquamation revealing the skin beneath to be of normal appearance. However, the gangrene of the toes and soles proved to involve the muscular layers as well as the skin. Preliminary amputation of the toes and of the devitalized tissues of the soles was performed on December 30, 1952, in an attempt to preserve the foot for weight bearing. However, it was soon evident that the necrotic tissue was too extensive and bilateral mid-calf amputations were performed, first on the right leg (January 13, 1953) and then on the left leg (February 11, 1953).

Examination of the amputated right leg revealed a normal appearing and widely patent dorsal pedal artery but a severely atherosclerotic posterior tibial artery. (Fig. 3A.) Portions of this vessel were yellow, hard and extremely narrow. The accompanying posterior tibial vein was thick-walled and two-thirds occluded by loose cellular tissue representing an organized thrombus. The amputated left leg showed no appreciable narrowing or hardening of the walls of either the dorsal pedal or posterior tibial artery. One large branch revealed an area of atherosclerosis with secondary calcification. (Fig. 3B.) Small arteries appeared to be normal.

The patient withstood the operations well and after a suitable interval bilateral prostheses were fitted and ambulation was restored. Cold agglutinins were not present in the blood on March 4, 1954. No determination had been done previously. Death occurred suddenly on October 3, 1954, and autopsy revealed old posterior and fresh anterior myocardial infarctions. Both femoral arteries showed atherosclerosis with moderate luminal narrowing.

COMMENTS

This case presented the striking paradox of an abrupt onset of bilaterally symmetric gangrene of the feet in the presence of readily palpable arterial pulsations. Also the color and temperature of the skin immediately adjacent to the



3A

3B

FIG. 3. Microscopic sections of vessels of amputated limbs. A, severely atherosclerotic right posterior tibial artery and thrombosis of right posterior tibial vein; B, atherosclerotic area in left posterior tibial artery (lower section). Note normal appearance of remainder of arterial wall (upper section).

gangrenous areas appeared normal. Symmetric gangrene of the legs can occur in association with a saddle embolus at the aortic bifurcation but this possibility appeared to be ruled out by the presence of bilaterally palpable femoral pulses. Similarly, the palpability of pulsations in the arteries of the feet was evidence against the possibility of simultaneous obliteration of peripheral arteries in both legs as the cause of the gangrene. On this basis it was concluded that the gangrene was not the result of arterial occlusion but was related to the prolonged peripheral ischemia associated with bradycardia and hypotension. Examination of the amputated legs supported this view by revealing incomplete arterial occlusion on one side, normally patent arteries on the other and uniformly normal appearing small vessels bilaterally.

Many different factors may be involved in the production of gangrene of the extremities and in individual cases more than one factor may be involved. The following is an outline of possible mechanisms concerned in the etiology of gangrene.

I. Changes in the external environment of the part
II. Changes in the internal environment of the part
  A. Infection
  B. Reduced oxygenation of tissues
    1. Ischemia
      a. Afferent (arterial) lesions
        1. Organic arterial occlusion
          a. Obliterative arterial disease
          b. Embolism
          c. Thrombosis
          d. Cryoglobulinemia
          e. Cold agglutinins
          f. Essential thrombocytosis
        2. Arterial spasm
          a. Raynaud's syndrome
          b. Circulatory shock
          c. Intoxications (ergot)
      b. Efferent (venous) lesions
        1. Venous thrombosis
    2. Anoxemia
      a. Anemia
      b. Carbon monoxide poisoning

Gangrene of the extremities is most commonly due to occlusion of the arteries supplying the affected part. Non-occlusive gangrene is symmetric and less common; it has been rarely reported in the literature. However, the available data are provocative and worthy of discussion in an attempt to elucidate the mode of development of gangrene. The various conditions which have been reported as exhibiting this phenomenon are: (1) congestive heart failure, (2) mitral ball-valve thrombus, (3) tight mitral stenosis, (4) myocardial infarction, (5) paroxysmal tachycardia, (6) pulmonary infarction, (7) circulatory collapse of unknown cause, (8) cholera, (9) pneumonia, (10) tuberculosis and (11) carbon monoxide poisoning.

As indicated in the foregoing, most of the cases reported have accompanied some form of cardiovascular disease.

In 1938 Fishberg[1] reported the phenomenon of the coexistence of collapsed veins in the extremities and engorged jugular veins in moribund patients with severe right ventricular failure. By means of direct simultaneous measurements of antecubital and external jugular venous pressures he demonstrated a fall in antecubital pressure occurring despite a persistently elevated jugular pressure. In extreme instances the jugular pressure exceeded 20 cm. of water, whereas the simultaneous antecubital pressure was below 1 cm. Improvement, which was exceptional, was accompanied by a rise in antecubital pressure to the level of the jugular pressure. In two cases he observed symmetric peripheral gangrene in association with this phenomenon. He interpreted the condition as a manifestation of reflex vasoconstriction in the extremities occurring in response to extreme reduction in cardiac output. This selective vasoconstriction results in a redistribution of blood, with more blood going to the brain and other vital organs at the expense of an ischemia of the limbs. In his textbook on heart failure Fishberg[2] discussed symmetric peripheral gangrene as a rare manifestation of cardiac shock, citing instances observed in cases of mitral stenosis with ball-valve thrombus, very tight mitral stenosis without ball-valve thrombus, and myocardial infarction. In each instance the development of peripheral gangrene accompanied the typical picture of cardiac shock, and postmortem examination revealed no evidence of arterial embolization or thrombosis.

Perry and Davie[3] in 1939 reported a case of symmetric gangrene of the legs in a sixty-four year old man exhibiting severe congestive heart failure. Autopsy revealed no obstruction of the iliac, femoral or popliteal arteries.

Swan and Henderson[4] in 1951 reported two cases of peripheral gangrene in myocardial infarction. Their first case was that of a man of fifty-three who had congestive heart failure, prolonged hypotension and gangrene of the tip of the nose and of both feet. Postmortem examination revealed an extensive recent myocardial infarction with a left ventricular mural thrombus. Dissection of the femoral, popliteal, peroneal and posterior tibial arteries as far as the ankles and the dorsalis pedis and lateral plantar arteries on both sides revealed no evidence of thrombus or of embolism, and there was no macroscopic evidence of intimal degeneration in these vessels. Their second case was that of a sixty-three year old woman with a history of Raynaud's disease in whom myocardial infarction developed accompanied by characteristic electrocardiographic changes and congestive heart failure; however, the blood pressure was within normal limits. Gangrene of the toes and fingers appeared, but the radial, dorsal pedal and posterior tibial pulsations remained palpable bilaterally. Griffith[5] has observed three cases exhibiting symmetric gan-

grene in association with peripheral vascular collapse in myocardial infarction. Pressor drugs were employed without apparent benefit.

Abrahams[6] reported the case of a forty-seven year old housewife who had ventricular paroxysmal tachycardia (rate 230/minute) which persisted for three days and was associated with hypotension, congestive heart failure and symmetric gangrene of the hands, feet, nose and ears. The neck veins were greatly distended and quivering but the arm veins were so empty of blood that they could not be seen at all. Death occurred and an autopsy showed no abnormalities of the arteries of the upper and lower extremities.

Hejtmancik and Bruce[7] have recently reported a case of massive pulmonary infarction associated with circulatory collapse and followed by extensive symmetric gangrene which necessitated bilateral leg amputations. Arterial pulsations in the feet remained normal until after the onset of the gangrene. Microscopic sections of the amputated limbs showed moderate arteriosclerotic narrowing of arteries. These vessels contained multiple organizing thrombi. The authors attributed the gangrene to severe peripheral vasoconstriction of arterioles developing as a reflex response to a marked fall in cardiac output and superimposed upon pre-existing vascular disease. Poor oxygenation of blood in the lungs due to the extensive pulmonary involvement was considered contributory.

de Silva[8] observed symmetric gangrene of the feet and legs in a forty-two year old man who expired after fourteen days of persistent hypotension and bradycardia. Blood pressure ranged from 80/56 to 80/60 and the pulse rate varied from 44 to 68 per minute. He suggested that the gangrene was caused by persistently low blood pressure and bradycardia.

The peripheral gangrene encountered in cardiovascular diseases appears to be related primarily to reduced cardiac output, reflex arterial spasm in the extremities with secondary venous collapse and anoxemia.

Non-occlusive symmetric gangrene has been reported in a number of infectious diseases.

Peripheral gangrene caused by the extreme vasoconstriction that accompanies severe circulatory failure in cholera is well recognized.[9]

A number of authors[10–13] have described symmetric gangrene complicating pneumonia and in the case reported by Dufour[10] no obliterative vascular lesion was found at autopsy.

Valtis and his associates[14] observed acute symmetric gangrene involving all four extremities and appearing during the terminal stage of bilateral pulmonary tuberculosis in a girl of fifteen. At the time of appearance of the gangrene the blood pressure was 95/60 and a moderate anemia (3.79 million erythrocytes per cu. mm.) existed. Autopsy revealed no vascular coagula and no lesions of the heart or blood vessels. Vischer[15] reported symmetric gangrene complicating a severe infectious disease with circulatory collapse. Meningococcemia was strongly suspected on circumstantial evidence but was not proved bacteriologically. The cases complicating acute and chronic infectious disease probably represent more than one mechanism. Reduced cardiac output and anoxemia, due to anemia or to reduced oxygenation of blood in the lungs, undoubtedly play important roles.

A number of authors[16–20] have reported instances of peripheral gangrene following exposure to carbon monoxide. Some have described acute arteritis and thrombosis. However, Boidin, in discussing the case of Laignel-Lavastine,[19] cited the development of gangrene of the extremities in five German soldiers overcome by carbon monoxide in a shelter. He attributed the gangrene to a combination of reduced oxygen supply and local pressure effects. Enzer and Spilberg[18] reported a case in which the patient had gangrene of one leg and only partial thrombotic occlusion of the corresponding popliteal artery. They suggested that anoxia had played an important role in causation of the gangrenous process. Thus in carbon monoxide poisoning the anoxemia would seem to be paramount but other factors, such as acute arteritis, probably are important.

Many of the cases of peripheral gangrene reported in the literature[21–24] have been unilateral or have shown arterial occlusion and yet are pertinent to the discussion since some of the factors so far mentioned played important roles in initiating the gangrenous condition. For example, Levine[21] refers to the case of a man of forty who was otherwise perfectly well and who had had 3 attacks of paroxysmal tachycardia over a period of several years. The three attacks were complicated in turn by a transient hemiplegia, a transient aphasia and dry gangrene of the left arm requiring amputation. During the attacks the blood pressure was 94–96 mm. Hg systolic and about 88 mm. Hg diastolic, the effective

pulse pressure thus being only 6–8 mm. Hg. Levine suggested thrombosis of peripheral vessels secondary to stagnation of blood as the cause of the gangrene and other sequelae.

Occlusive venous disease as well as arterial disease may result in gangrene, as pointed out by Haimovici[25] who has recently reviewed the literature concerning the rare entity of gangrene of the extremities of venous origin occurring in association with acute thrombophlebitis. He suggested the initiating and basic cause to be a complete blockage of the venous system in the involved extremity with resulting tissue anoxia. Two of the twenty-nine cases he reviewed showed symmetric gangrene.

In conclusion, analysis relative to the cause of symmetric, non-occlusive gangrene reveals that only three factors appear to be of etiologic significance, namely, prolonged hypotension, reduced cardiac output and anoxemia. None of these could represent a single basic factor explaining the gangrene in all cases, since none was a constant feature of all cases. However, all three result in reduced oxygenation of the peripheral tissues.

SUMMARY

A review of the literature reveals that the rare phenomenon of non-occlusive, acute, symmetric peripheral gangrene may complicate a variety of clinical conditions exhibiting prolonged hypotension, reduced cardiac output, anoxemia or various combinations of these, all resulting in marked and prolonged hypoxia of tissues in the extremities. The condition may be clinically diagnosed in patients exhibiting one or more of the above factors and developing symmetric gangrene in extremities which show normal arterial pulsations.

REFERENCES

1. Fishberg, A. M. Redistribution of blood in heart failure. *J. Clin. Investigation*, 17: 510, 1938.
2. Fishberg, A. M. Heart Failure, 2nd ed., p. 656. Philadelphia, 1940. Lea & Febiger.
3. Perry, C. B. and Davie, T. B. Symmetrical peripheral gangrene in cardiac failure. *Brit. M. J.*, 1: 15, 1939.
4. Swan, W. G. A. and Henderson, C. B. Peripheral gangrene in myocardial infarction. *Brit. Heart J.*, 13: 68, 1951.
5. Griffith, G. C. Personal communication.
6. Abrahams, D. G. Incipient symmetrical peripheral gangrene complicating paroxysmal tachycardia. *Brit. Heart J.*, 10: 191, 1948.
7. Hejtmancik, M. R. and Bruce, E. I. Symmetrical peripheral gangrene complicating pulmonary embolism. *Am. Heart J.*, 45: 289, 1953.
8. de Silva, S. Symmetrical peripheral gangrene with low blood pressure. *J. Ceylon Br., Brit. M. A.*, 36: 373, 1939.
9. Strong, R. P. Stitts' Diagnosis, Prevention, and Treatment of Tropical Diseases, 6th. ed., p. 621. Philadelphia, 1942. The Blakiston Co.
10. Dufour, H. Note sur la pathogenie des gangrenes symetriques des extremities dans les infections pneumoniques, à propos d'un cas autopsie. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 18: 1065, 1901.
11. Uhr, J. S. Gangrene of the toes following pneumococcemia. *Am. J. Dis. Child.*, 56: 1317, 1938.
12. Chatterjee, S. C. Symmetrical peripheral gangrene in lobar pneumonia. *Calcutta M. J.*, 37: 221, 1940.
13. Storstein, O. Incipient symmetrical peripheral gangrene complicating pneumonia, case report. *Brit. Heart J.*, 13: 411, 1951.
14. Valtis, J. S., Catacouzinos, A., Caccavias, S. and Chrysanthoy, C. Gangrene symetrique des membres chez une tuberculeuse a la periode terminale de sa maladie. *Presse méd.*, 56: 171, 1948.
15. Vischer, W. Symmetrische gangrän nach infecktionskrankheiten. *Helvet. méd. acta*, 18: 422, 1951.
16. Wilson, J. A. and Carey, W. C. Gangrene of a foot following carbon monoxide poisoning. *Indust. Med.*, 9: 197, 1940.
17. Cranmer, R. R. Gangrene of the foot following carbon monoxide poisoning; report of a case. *Minnesota Med.*, 27: 934, 1944.
18. Enzer, N. and Spilberg, S. Gangrene of lower extremity following carbon monoxide asphyxia. *Am. J. Clin. Path.*, 16: 111, 1946.
19. Laignel-Lavastine and Alojouanine. Illuminative gas poisoning followed by dry gangrene and phlegmasia alba dolens. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 484, 1921.
20. Fowler, P. B. S. Gangrene of the leg following carbon-monoxide asphyxia. *Lancet*, 266: 240, 1954.
21. Levine, S. A. Clinical Heart Disease, p. 191. Philadelphia and London, 1954. W. B. Saunders Co.
22. Boquien, Y., Hervouet, D., Dauphin, G. and Verdier. Polygangrene aigue symetrique au cours d'une endocardite maligne. *Presse méd.*, 60: 1581, 1952.
23. Tölle, K. H. Extremitätengangränen als Folge eines kongenitalen Herzfehlers. *Frankfurt Ztschr. Path.*, 64: 1, 120, 1953.
24. Weiner, H. A. Gangrene of the extremities. A recently recognized complication of severe meningococcic infection. *Arch. Int. Med.*, 86: 877, 1950.
25. Haimovici, H. Gangrene of the extremities of venous origin; review of the literature with case reports. *Circulation*, 1: 225, 1950.

Hamman-Rich Syndrome*

Report of a Case Diagnosed Antemortem by Lung Biopsy and Successfully Treated with Long-term Cortisone Therapy

C. T. PINNEY, M.D. *and* H. WILLIAM HARRIS, M.D.

Denver, Colorado *Salt Lake City, Utah*

In 1935 Hamman and Rich[1] first described a fatal pulmonary disease of unknown etiology which has since become known as the Hamman-Rich syndrome. Twenty-seven cases have been reported since the original description;[2] however, the number reported in recent literature indicates an increased awareness of this disease.[3] This report will detail a case of Hamman-Rich syndrome diagnosed antemortem by lung biopsy and successfully treated for eight months with cortisone.

The etiology of this disease remains obscure although many theories have been proposed. Originally it was thought to be related to a virus infection or a hypersensitivity reaction.[1,4] Recently, interstitial fibrosis of the lung has been reported to occur during apresoline® therapy for hypertension.[5] Some observers[6] believe this type of pulmonary fibrosis may be the sequel to one or more attacks of acute interstitial pneumonia which heals by organization. Silverman and Talbot[7] suggest that this syndrome is not a specific disease but a non-specific host reaction initiated by a variety of agents. Interstitial fibrosis of the lungs occurs not only in this syndrome but also following attacks of influenzal pneumonia, scrub typhus and other rickettsial pneumonias, or following exposure to various chemical irritants. In the majority of cases, however, the etiology of the Hamman-Rich syndrome cannot be established.

The clinical manifestations vary from case to case; most patients have a dry cough, dyspnea and cyanosis of varying degree and severity. In some instances the disease is acute, with sudden onset and rapid progression to death from respiratory insufficiency. In other cases there is a more chronic course with gradual downward progression and eventual death from cardiac failure. The shortest duration of the disease reported has been thirty-one days;[4] the longest reported, nine years.[3] There are varying opinions as to the natural history of the disease. Rubin[8] indicates that in the majority of cases the onset is acute. Golden and Brock[6] believe the onset may be subclinical with slowly progressive disease, and that the symptomatic phase is, in reality, the terminal stage of the illness. Hemoptysis, chest pain and weakness have been described occasionally in this syndrome. Fever may be present but it usually occurs terminally. In spite of marked symptoms the physical findings are usually minimal. Cyanosis may be present in varying degrees, depending on the stage and severity of the illness, but it is usually present terminally. Pulmonary osteoarthropathy may occur. In long-standing, chronically progressive cases, signs of right-sided cardiac failure are seen.

The laboratory findings are not specific. The leukocyte count is usually normal but leukocytosis may occur in the terminal phase. Erythrocytosis is occasionally seen. Tests of cardiopulmonary function in one patient studied by Silverman and Talbot[7] revealed a normal tidal volume and ventilatory capacity. The cardiac output was increased, the right ventricular and pulmonary artery pressures were elevated, and arterial oxygen unsaturation was present. On the basis of their studies these observers believed the physiologic defect in their case to be "alveolocapillary" block and this view is widely held.

The roentgenographic appearance is not specific. The lungs usually show evidence of extensive infiltration. This may appear miliary, reticular, nodular or coalescent in character. Occasionally, small pleural effusions are seen. In the late stage of the disease the infiltration becomes more extensive and shows a tendency

* From the Department of Medicine, Fitzsimons Army Hospital, Denver, Colorado.

to increased confluency. Hilar lymphadenopathy may be present.

Surprisingly little if any knowledge of the pathologic changes has been added since the original description. Grossly, the lungs are firm and rubbery, and sink in water. Emphysematous blebs may be present on the pleural surfaces. The lungs cut with increased resistance and present a gray to red-brown granular surface which is spanned by a grayish white fibrous network. Hamman and Rich[1,4] described four stages.

Stage I: A widespread inflammatory reaction characterized by albuminous fluid in the alveoli, together with a few red blood cells and, often, polymorphonuclear leukocytes. The lining cells of the alveoli are often enlarged and may be desquamated, and a hyaline membrane often lines the desquamated areas. No bacteria are seen. In some areas proliferation of fibroblasts is seen in the alveolar walls. There may be small foci of early organization of hemorrhage in the alveoli.

Stage II: This stage is characterized by progressive proliferation of fibroblasts in the thickened alveolar walls which may encroach upon or obliterate the alveolar spaces. Slight organization of the intra-alveolar exudate may be seen. The pathologic process varies in that some areas show newly formed fibroblasts and other areas demonstrate dense hyaline connective tissue. Inflammatory cells are inconspicuous. Some of the alveoli are lined with enlarged epithelial cells and some with a hyaline membrane. The process is seen in all lobes of both lungs. Eosinophils may be present.

Stage III: This is similar to Stage II except that the fibrous tissue is more mature. In some areas early fibroblastic proliferation may be seen.

Stage IV: The fibrous tissue is mature and no early fibroblastic proliferation is observed.

Recently, Golden and Brock[6] examined the pulmonary tissue of three fatal cases of Hamman-Rich syndrome using phase microscopy and Schiff periodic acid stain. They demonstrated an extreme degree of capillary proliferation in the alveolar walls and hyperplasia of reticulum fibers in this area. Focal collections of collagenous stroma were observed. They termed this change "reticulo-angiosis" and believe these abnormalities responsible for the alveolar septal hypertrophy.

In the past the diagnosis has been established by autopsy in the majority of instances and has been suspected clinically in few cases. Thoracotomy and lung biopsy have established the diagnosis in three cases,[3,7,11] in addition to the present one.

Treatment in the past has been unsuccessful and, until recently, largely symptomatic. The use of antibiotics and other chemotherapeutic agents has not altered the course of the disease. The use of cortisone and corticotrophin has been reported in eight cases. Callahan et al.,[9] Cox and Kohl,[10] and Schechter[11] used corticotrophin without benefit in three cases. Silverman and Talbot[7] treated a patient with cortisone for two months, noted moderate symptomatic improvement for two weeks, but observed no change on x-ray of the chest. The disease progressed and the patient died of cardiac failure. Peabody[3] treated one patient with intravenous corticotrophin, 15 mg. daily; when improvement occurred the dose was decreased to 10 mg. and, after maximal response, oral cortisone, 100 mg. daily, was used. The patient remained essentially symptom-free for two months and then cortisone was gradually withdrawn and adrenal-stimulating doses of corticotrophin were given. Nine days after cessation of cortisone a marked symptomatic and radiologic relapse occurred and, in spite of large doses of cortisone and corticotrophin, the patient died of acute respiratory insufficiency within twenty-four hours. The second patient treated by this group with increasing doses of intravenous corticotrophin (25–50 mg.) noted symptomatic improvement but when the dose was gradually decreased to 30 mg. daily marked exacerbation of symptoms occurred. The patient died in spite of an increase in the intravenous dose of corticotrophin to 50 mg. and the addition of 300 mg. of cortisone daily. A third patient was given cortisone alone, 75 mg. daily by mouth, for three weeks. No radiologic change was noted but the patient improved symptomatically. Cortisone therapy was stopped abruptly and after seven days a marked exacerbation of symptoms recurred. The patient died of pulmonary insufficiency. Schechter[11] noted marked symptomatic and radiologic improvement in one patient given corticotrophin intravenously, 25 mg. daily for three weeks. Cortisone, 200 mg. orally, was then begun and corticotrophin was discontinued. The dose of cortisone was gradually decreased to 100 mg. daily and maintained at this level for two months. Improvement per-

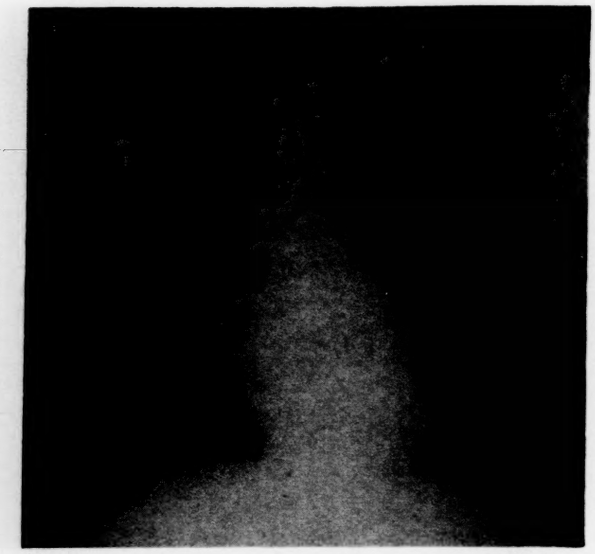


FIG. 1. X-ray of the chest four months after onset of symptoms and two months prior to cortisone therapy.

FIG. 2. Photomicrograph of lung tissue obtained at thoracotomy.

sisted for this period but then the dose of cortisone was gradually decreased and corticotrophin added to stimulate adrenal activity. The patient remained well during the withdrawal period and for four days after discontinuation of all therapy. Then a marked symptomatic and radiologic relapse recurred and the patient died of acute respiratory insufficiency in spite of large doses of cortisone and corticotrophin during the last twenty-four hours of life.

CASE REPORT

A twenty-seven year old housewife was admitted to Fitzsimons Army Hospital on January 21, 1954. She had been free of respiratory symptoms until September 1, 1953, when she noted the onset of a cough productive of mucoid sputum, and moderate dyspnea. A twenty-pound weight loss had occurred during the previous nine months. For a few days she had noted vague left anterior chest pain aggravated by coughing. X-ray of the chest on September 10th revealed widespread infiltration in both lungs, and she was admitted to the Station Hospital, Wright-Patterson Air Force Base, where investigation was undertaken. Symptoms continued and since a definite diagnosis could not be established she was transferred to Fitzsimons Army Hospital. Examination revealed the patient to be a well developed, poorly nourished white woman who was slightly dyspneic at rest and who coughed spasmodically. Blood pressure was 105/80 mm. Hg, pulse rate 92 and respiratory rate 24. There was no fever. Physical examination was normal and no abnormal physical findings were noted on examination of the lungs.

Cardiolipin microflocculation, complete urinalysis, blood urea nitrogen, serum proteins, serum bilirubin, cephalin cholesterol flocculation and thymol turbidity tests, prothrombin time, serum chloride, serum potassium and fasting blood sugar were all normal. A culture of the sputum on blood agar plates revealed Streptococcus viridans on one occasion and Candida albicans on other cultures. Cultures of the sputum, gastric and bronchial washings, urine and bone marrow for tubercle bacilli were negative. Cultures of bronchial washings for fungi were negative. Skin tests were positive to tuberculin PPD (0.00002 mg.) and histoplasmin antigen (dilution 1:100). The skin test was negative for coccidioidin antigen (dilution 1:100). A complement fixation test for psittacosis and lymphogranuloma antibodies was negative. Complement fixation tests for histoplasmosis antibody were positive in a dilution of 1:32 on January 28, 1954; 1:16 on February 8, 1954; but negative on March 31, 1954. The leukocyte count was 11,000 and 12,000 on two occasions but normal on several other occasions. Hemoglobin and hematocrit determinations were normal. The vital capacity was 2.7 L. (predicted

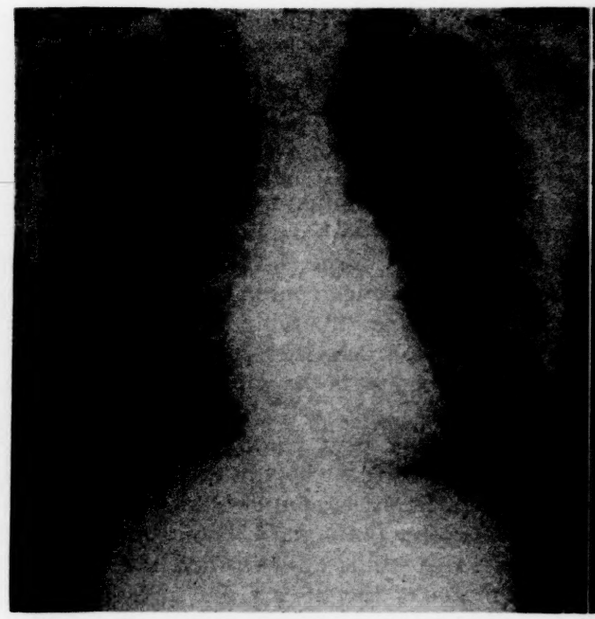


FIG. 3. X-ray of the chest two and one-half months after initiation of cortisone therapy.

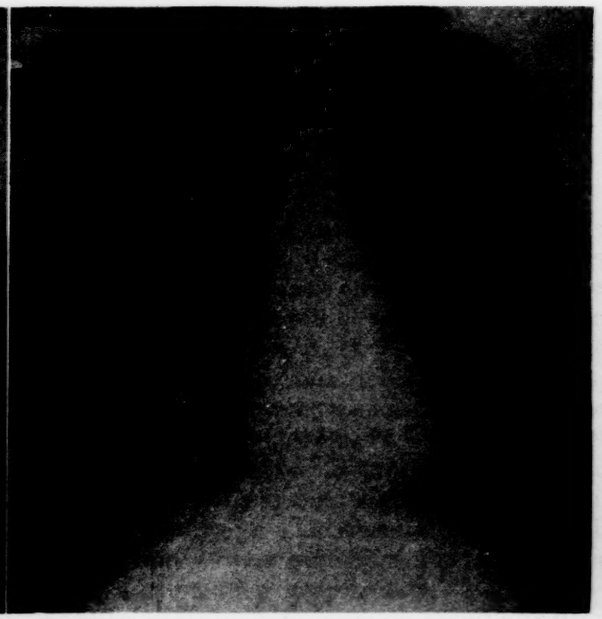


FIG. 4. X-ray of the chest after eight and one-half months of cortisone therapy.

value 2.8 L.). The expiratory volume during the first second was 2.0 L. An electrocardiogram was normal. A bone marrow aspiration revealed normal cellular elements. Bronchoscopy showed no abnormalities.

X-ray of the chest taken in July, 1953, three months prior to onset of symptoms, was reviewed and interpreted as normal. X-ray on admission to Fitzsimons Army Hospital (Fig. 1) revealed extensive pulmonary infiltrates of a lacy and reticular appearance throughout both lungs. Fine and coarse nodularity was apparent, and there was evidence of slight enlargement of the hilar lymph nodes.

On March 2, 1954, punch biopsy of the liver showed no abnormalities.

Because an etiologic diagnosis could not be established, a left thoracotomy was performed on March 8, 1954. The lung appeared normal to inspection but on palpation felt granular. A few enlarged lymph nodes were noted in the hilar region. Microscopic examination of the pulmonary tissue (Fig. 2) revealed severe diffuse interstitial fibrosis, some of which appeared quite active and proliferative while in other regions hyalinized, relatively acellular scar tissue was seen. Complete replacement of the alveoli was present in some areas. Some of the alveoli was lined by a hyaline-type membrane and others contained hemorrhage and edema fluid. Macrophages singly and in clumps were seen within many alveoli. The inflammatory infiltrate was composed of lymphocytes, a moderate number of polymorphonuclear leukocytes and eosinophils. Cultures, smears and tissue stains were negative for acid-fast bacilli, fungi and bacteria. The pathologic diagnosis was diffuse interstitial pulmonary fibrosis similar to that described by Hamman and Rich.

On March 24, 1954, the patient was given 200 mg. of cortisone orally, together with penicillin 300,000 units and streptomycin 1.0 gm. daily by the intramuscular route. Within forty-eight hours the patient noted a marked improvement in the cough and dyspnea. On April 14, 1954, a severe tearing pain developed in both lower extremities, the cause of which was not apparent. Cortisone was omitted for forty-eight hours with complete relief of pain. The drug was resumed in a dose of 150 mg. daily on April 16th. The following day the dose was reduced to 100 mg. per day, and on May 28, 1954, it was further reduced to 75 mg. per day. Serial x-rays of the chest demonstrated marked clearing of the infiltrates. (Fig. 3.) The patient was discharged from the hospital on June 8, 1954, but continued on a regimen of 75 mg. oral cortisone daily, penicillin, 400,000 units orally twice daily, potassium chloride, 1.0 gm. twice daily, as well as a salt restricted diet. This regimen was continued on an outpatient basis during July and August without symptomatic or roentgenographic relapse. On September 1, 1954, gradual reduction of cortisone was begun by alternating doses of 75 mg. and 50 mg. daily. After one week the dose was maintained at 50 mg. daily, which has been continued to the present time without complications. Frequent x-rays of the chest during the follow-up period have shown continued improvement and the most recent film of December 8, 1954, is virtually normal. (Fig. 4.) The patient has been seen in the outpatient clinic

twice each month. Eight and one-half months after initiation of treatment with oral cortisone, she is asymptomatic and carries on relatively normal activities.

COMMENT

The clinical, laboratory, radiologic and pathologic findings in this patient appear typical of the Hamman-Rich syndrome. Because x-ray of the chest was essentially negative three months prior to the initial symptoms, the onset of the illness can be fairly well established. The positive skin reaction and complement fixation test for histoplasmosis deserve comment. Although the patient lived in an area known to be endemic for this fungus, pathologic examination of the lung biopsy specimen excluded this disease. The intradermal injection of histoplasmin antigen for sensitivity testing has been reported to stimulate a complement fixing antibody rise in certain individuals who demonstrate a positive skin reaction.[12] Two strongly positive skin tests were performed prior to the demonstration of complement fixing antibodies in this patient. It is possible that this phenomenon occurred here.

Neither the clinical picture nor the radiographic findings are specific in Hamman-Rich syndrome. Differentiation of this disease from other chronic lung diseases by clinical evaluation alone is usually difficult or impossible. Often it may closely resemble tuberculosis, fungus infections, pneumonconiosis, sarcoidosis, parasitic infestations, eosinophilic pneumonopathy or lymphangitic dissemination of metastic carcinoma. A clinical diagnosis of Hamman-Rich syndrome can be made only by exclusion of other chronic diseases which cause diffuse pulmonary infiltrates. A proved diagnosis during life can be established only by lung biopsy, which when accomplished by a competent thoracic surgeon carries little risk. A positive biopsy specimen provides a specific diagnosis and an intelligent basis for formulation of prognosis and treatment.

Heretofore, all reported forms of treatment have been unsuccessful in this disease. There have been eight reported cases of patients treated with corticotrophin, cortisone or both.[3,7,9–11] It is noteworthy that the two patients demonstrating regression of the pulmonary lesions were treated soon after the onset of symptoms as was the present case.[3,11] The remainder who did not respond had symptoms over a longer interval prior to therapy. Hamman and Rich noted originally that the early stages of the disease were associated with a proliferative inflammatory and fibroblastic reaction, whereas in the late stages mature fibrous tissue was present. Cortisone has been reported to inhibit the formation of inflammatory exudates and granulation tissue but to have little or no effect upon mature fibrous tissue.[13] It is on this basis, we believe, that corticotrophin or cortisone therapy may produce a favorable trend in the early stages of Hamman-Rich syndrome but offer little or no benefit in the late stages of the disease.

Possibly, Hamman-Rich syndrome may exist in a less severe form, compatible with an excellent prognosis. In such cases therapy may not be indicated. However, patients with symptoms or with extensive pulmonary lesions have a serious prognosis. It would appear, therefore, that early recognition of the disease and prompt institution of treatment are important if any hope is to be offered patients with this disease. Considerable significance must be attached to the pathologic stage of the disease in planning therapy. It is not clear how long therapy should be continued once it has been initiated. The two reported cases who demonstrated a favorable trend during therapy suffered prompt and violent relapse and death when treatment was discontinued. The patient reported here has been receiving continuous therapy for eight months; she appears to tolerate the present dose of cortisone without evidence of the unfavorable reactions reported to occur with long-term therapy.[13] The serious risk of death from the chronic lung disease, we believe, outweighs the hazard of long-term cortisone therapy. However, it will be necessary for the patient to be under careful medical observation as long as this treatment is continued.

The favorable response to treatment in the early stages of the disease and immediate relapse on cessation of treatment which has been reported suggests that adrenal steroid therapy does not remove the etiologic stimulus to the inflammatory and fibroblastic reaction within the lung, but rather suppresses the host response to this stimulus. It is hoped that further study of this disease may lead to knowledge of the specific etiology and to some form of curative therapy. However, until such treatment is available suppression of the host response with corticotrophin or cortisone may provide a clinical remission.

SUMMARY

1. A case of Hamman-Rich syndrome diagnosed by lung biopsy is presented.

2. The patient is symptomatically well and x-ray of the chest is virtually normal eight and one-half months after initiation of continuous cortisone therapy.

3. This case demonstrates the longest clinical remission induced by therapy reported in the literature.

ADDENDUM

At the present time the patient remains clinically well after seventeen months of cortisone therapy. The dose of cortisone is 40 mg.

REFERENCES

1. Hamman, L. and Rich, A. R. Fulminating diffuse interstitial fibrosis of the lungs. *Tr. Am. Clin. & Climatols A.*, 51: 154–163, 1935.
2. Peabody, J. W. and Peabody, J. W., Jr. Diffuse fibrosing interstitial pneumonitis (Hamman-Rich syndrome). *Dis. of Chest.*, 26: 709–711, 1954.
3. Peabody, J. W., Jr., Buechner, H. A. and Anderson, A. E. Hamman-Rich syndrome: analysis of current concepts and report of three precipitous deaths following cortisone and corticotrophin withdrawal. *Arch. Int. Med.*, 92: 802–824, 1953.
4. Hamman, L. and Rich, A. R. Acute diffuse interstitial fibrosis of the lungs. *Bull. Johns Hopkins Hosp.*, 74: 177–204, 1944.
5. Morrow, J. D., Schroeder, H. D. and Perry, H. M., Jr. Studies on the control of hypertension by hyphex; II. Toxic reactions and side effects. *Circulation*, 8: 829–839, 1953.
6. Golden, A. and Brock, T. Diffuse interstitial fibrosis of the lungs. *Arch. Int. Med.*, 96: 606–614, 1953.
7. Silverman, J. J. and Talbot, T. Diffuse interstitial pulmonary fibrosis camouflaged by hypermetabolism and cardiac failure. *Ann. Int. Med.*, 38: 1326–1338, 1953.
8. Rubin, E. H., Kohn, B. S. and Peckar, D. Diffuse interstitial fibrosis of the lungs. *Ann. Int. Med.*, 36: 827–844, 1952.
9. Callahan, W. P., Sutherland, J. C., Fulton, J. K. and Kline, J. R. Acute diffuse interstitial fibrosis of the lungs. *Arch. Int. Med.*, 90: 468–482, 1952.
10. Cox, T. R. and Kohl, J. M. Diffuse fibrosing interstitial pneumonitis. *Am. Rev. Tuberc.*, 68: 603–614, 1953.
11. Schechter, M. M. Diffuse interstitial fibrosis of the lungs. *Am. Rev. Tuberc.*, 68: 603–614, 1953.
12. Saslow, S. and Campbell, C. C. Effect of Histoplasmin skin testing on serologic results. *Proc. Soc. Exper. Biol. & Med.*, 82: 689, 1953.
13. Thorn, G. W., Jenkins, D., Lordlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P. and McCracken, B. H. Pharmocologic aspects of adrenocortical steroids and ACTH in man. Medical progress report. *New England J. Med.*, 248: 284–294; 323–335; 369–378, 1953.

Familial Non-hemolytic Icterus*

WADDY G. BARODY, M.D. *and* MAJ. RICHARD T. SHUGART, M.C.†
Florence, South Carolina

FAMILIAL non-hemolytic icterus is recognized as a distinct entity.[3] It does not occur commonly; however, the exact incidence is not determined.[1,6] In the past it has been referred to as constitutional hepatic dysfunction.[1,2,6] In most instances the diagnosis is inadvertently arrived at after analysis of a patient with jaundice has excluded other etiologic factors.

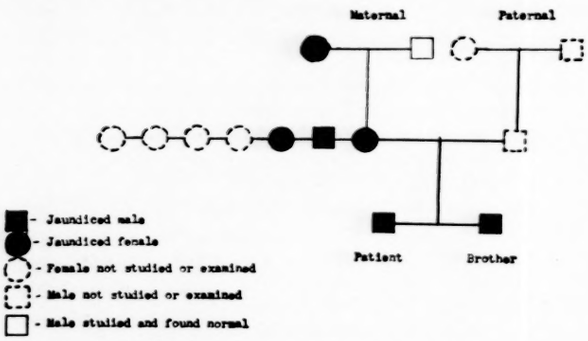


FIG. 1. Family tree of subject patient.

This report concerns a patient referred to us for evaluation of jaundice. Study revealed an icteric patient relatively asymptomatic and without evidence of anemia, hemolytic tendency or intrinsic liver disease. Members of three generations of his family were studied because intermittent jaundice had been observed in several individuals on the maternal side.

CASE REPORT

A nineteen year old white male was admitted to Fitzsimons Army Hospital July 26, 1954, because of jaundice. A few days prior to admission the patient was told by friends that he looked yellow and was advised to seek medical attention. He stated that he had been quite well and working every day but noted a mild degree of easy fatigability and some slight loss of appetite. Upon questioning he stated that his family had observed that he had had yellow skin from time to time ever since childhood. He related that on March 3, 1954, after having donated blood, he was told that his blood tests showed jaundice. The patient stated that he had always been entirely well without symptoms of nausea, vomiting, diarrhea, abdominal pain or fever. There had been no significant change in the color of urine or feces during this or any previous episode of observed jaundice.

Past history revealed that the patient's pre-military occupation was assistant funeral embalmer. His military history began October 13, 1953, at Portland, Oregon, thirty miles from his home. He had served only in the Continental United States.

The patient stated that he did not smoke or drink alcoholic beverages. No history of chronic drug intake or association with hematopoietic or hepatic toxic agents was obtained. Specific and detailed questioning revealed no evidence of inoculation or injection during the preceding several months with the exception of the previously mentioned blood donation. There had been no known exposure to contaminated food or water sources and no associates had been ill from infectious or contagious diseases. While in the military service the patient had been and is now serving as a surgical technician. (Fig. 1.)

Family history revealed that intermittent jaundice had been noted on the maternal side only. The patient's grandmother, mother, an uncle and an aunt were reputedly involved all of their lives with "yellow skin" at one time or another. The patient's brother and only sibling was not observed to have jaundice, although an elevated serum bilirubin was obtained. The paternal side of the family had no history of jaundice, liver or blood diseases. Both the maternal and paternal grandparents are living and well. No member of the family mentioned had had serious illnesses that required medical attention. The jaundice observed never stimulated a visit to the family physician.

This patient had been hospitalized only once, for "three day measles" in November, 1953. The only operations recorded in the history were a tonsillectomy and adenoidectomy at age five and a lipoma removed from the left hip during hospitalization at Fitzsimons Army Hospital.

On physical examination the temperature, pulse, respiration and blood pressure were within normal limits. A slight scleral icterus was noted but there was no evidence of liver palms, spider angiomas, abnormal hair distribution or gynecomastia. Examination of the

* From the Department of Medicine, Fitzsimons Army Hospital, Denver, Colorado.
† Presently on duty at the 6110th USAF Hospital, A.P.O., San Francisco, Calif.

TABLE I

LABORATORY DATA OF PATIENTS WITH JAUNDICE

	Indirect Serum Bilirubin	Total Serum Bilirubin	Red Blood Cell Count	Hemoglobin	White Blood Cell Count	Reticulocyte Count	Red Blood Cell Morphology	Red Blood Cell Fragility	Red Cell Sickling	Coomb's Test	Blood Type	History Clinical Jaundice
Patient	3.3	3.8	5.3	15.6	5100	0.7	Normal	Normal	Negative	Negative	O Rh+	Yes
Brother	2.7	2.99	5.7	15.8	5500	0.5	.....	.....	.....	.....	.....	No
Mother	1.15	1.35	3.85	14.3	7450	1.0	.....	Normal	Negative	Negative	O Rh−	Yes
Maternal aunt	1.15	1.30	3.74	14.1	7350	0.9	.....	Normal	Negative	Negative	O Rh−	Yes
Maternal uncle	3.77	3.77	5.46	15.5	6400	0.6	.....	.....	.....	.....	.....	Yes
Maternal grandmother	0.7	0.7	4.82	11.9	6500	0.4	.....	.....	.....	.....	.....	Yes
Maternal grandfather	0.29	0.49	....	....	....	....	.....	.....	.....	.....	.....	No

abdomen revealed a liver which could be palpated on deep inspiration but was not believed to be enlarged or tender. The spleen was not enlarged. The remainder of the physical examination was within normal limits.

Laboratory data revealed a red blood count of 6.2 million, hemoglobin 17.4 gm., white blood count 6,300 cells with a normal differential, sedimentation rate 3 mm. per hour, hematocrit 54. The blood morphology and urinalysis were within normal limits. The initial reticulocyte count was 1.2 per cent, subsequent counts of 0.7, 0.5 and 0.6 per cent were obtained. Coombs' test, sickle cell preparation and serologic tests for syphilis gave negative results. Two red cell fragility studies were entirely normal. Serum bilirubin was 3.7 mg. per cent, the direct van den Bergh test 0.5 mg. per cent, indirect 3.3; the total serum cholesterol was 144 mg. per cent, esters 79 per cent; serum alkaline phosphatase was normal, cephalin flocculation and thymol turbidity tests as well as BSP retention test and prothrombin time were within normal limits. A quantitative estimation of urine urobilinogen revealed 0.59 mg. per 24 hours, studies, over a three-day period, of fecal urobilinogen revealed an average excretion of 164.4 mg. per day. A urine test for porphyrins gave negative results. The patient's blood type was O Rh-positive. Bone marrow aspiration from the right iliac crest revealed normal tissue.

A radiochromium study of red cell survival time was reported as entirely within normal limits and paper chromatography revealed normal adult hemoglobin. The serum bilirubin remained elevated, only the indirect component being abnormal. Evidence collected revealed no hepatic or hemolytic blood disease. A bilirubin tolerance test was done which revealed 83 per cent retention four hours after a test dose of 50 mg. of bilirubin had been administered intravenously, as compared with normal retention of less than 5 per cent. Total evaluation of the data presented led us to the conclusion that this represented a typical case of familial non-hemolytic icterus.

Physical examinations and some laboratory studies were made of the patient's mother and aunt. In addition, relevant laboratory data were obtained on the patient's brother, uncle and both grandparents. (Table I.) The histories obtained agreed in all details in regard to family illness and the observations regarding intermittent jaundice. Physical examinations on the mother and aunt were entirely normal, no evidence of clinical icterus or enlargement of the spleen or liver being obtained. From the data collected it appeared that there was familial transmission of icterus on the maternal side of the family.

COMMENTS

Familial non-hemolytic icterus has been recognized as an entity for many years. In past instances the familial jaundice reported usually represented the hemolytic form, as previously recognized,[3,10] and this had to be strongly considered in the differential diagnosis of our patient. The diagnosis requires (1) familial occurrence of jaundice, (2) an elevated serum bilirubin reflected in the indirect component, (3) no evidence of anemia, (4) no apparent

hemolytic blood disease, (5) no liver disease, and (6) a jaundiced yet asymptomatic patient in otherwise good health.

The case presented fulfills these criteria. The patient was jaundiced yet essentially asymptomatic. Physical examination revealed jaundice to be the only significant abnormality. Laboratory data revealed an elevated indirect and total serum bilirubin. This suggested the possibility of hemolysis. Study showed no anemia and no evidence of reticulocytosis or sickling, or any increase in excretion of fecal and urinary urobilinogen. Bone marrow aspiration, erythrocyte fragility, red cell survival time, Coombs' test and paper chromatography were normal. These findings militated against abnormal hemolysis.

Liver function studies were performed and no abnormalities discovered. The bilirubin tolerance test revealed delayed clearance. No evidence of acute or chronic disease was apparent.

Familial occurrence by history and laboratory data is in accord with previously described cases. The absence of increased serum bilirubin in the maternal grandmother we believe represents the intermittent manifestation of icterus since the family noted "darkening of her skin" from time to time.

The most frequently described symptom in previous reports is easy fatigability.[1,6,7] There is no pain or fever, and the relationship of jaundice to physical activity has been a point of conjecture; no specific correlation was noted in our patient. Emotional stress has been related to icterus by some observers but this has not been applicable to our patient or to involved members of his family.

The mechanism appears to be a physiologic defect resulting in impaired clearance of the serum bilirubin.[3,7] This probable defect is illustrated and substantiated by the results of the bilirubin tolerance test recorded in the case report, in addition to all the other data presented.[3–5] This defect is probably a dominant mendelian trait which may be carried by either sex.[3,8]

The prognosis in patients with familial nonhemolytic icterus is excellent.[3,6,7] There is no evidence of abnormal red blood cell destruction or primary hepatic damage and no evidence of progression of the disease state as there might be in chronic hepatic disease. The patient reported in this study is age nineteen but his entire family including mother, aunt, uncle and grandparents are still living a normal life into the seventh decade; therefore age seems not to be a factor except perhaps as an indicator that the prognosis is very good. We are not sure whether the easy fatigability has anything specifically to do with the disease; it may, of course, reflect the personality of the individual involved. Whether or not infection or physical stress have any bearing on the degree of jaundice has yet to be determined.

SUMMARY

A study was made of three generations of a family each presenting a history of jaundice, substantiated by laboratory data.

The family members were active and in good health. No evidence of hemolysis was unearthed even though indirect-reacting bilirubin of the serum was the abnormal component in all affected members. No evidence of hepatocellular damage was obtained. These findings support the diagnosis of non-hemolytic icterus exhibiting familial transmission, in this instance on the maternal side of the family.

The importance of recognition of this established entity is stressed. The prognosis is excellent and confusion with congenital hemolytic anemia or chronic liver disease could well be the source of iatrogenic illness.

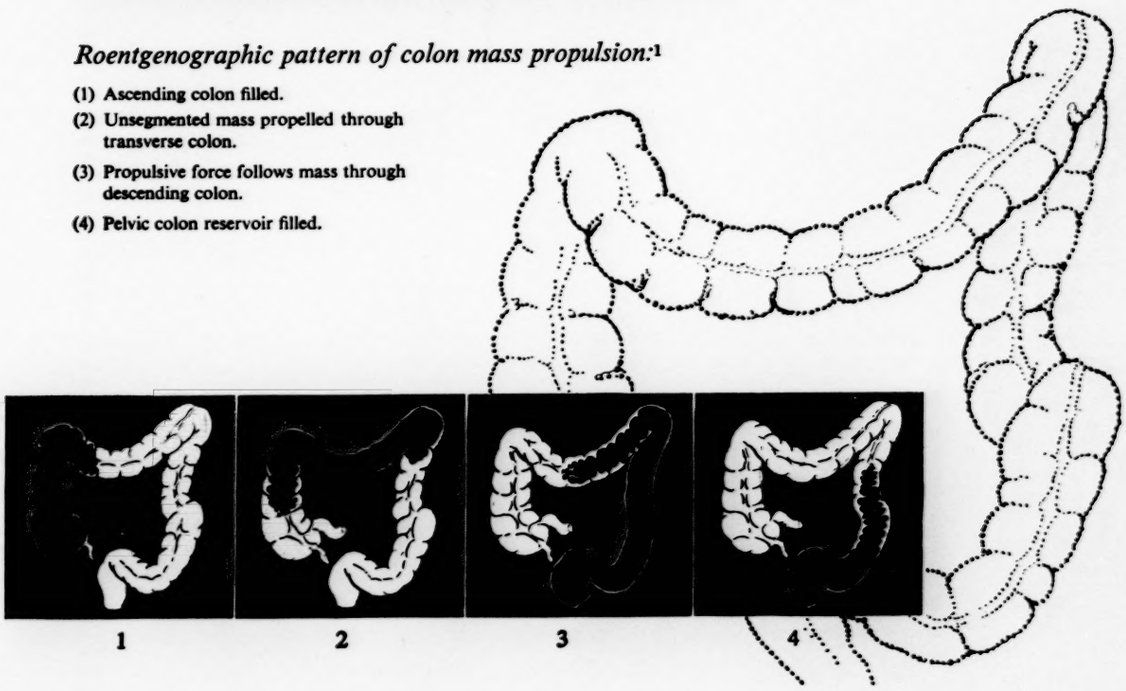
REFERENCES

1. Comfort, M. W. Constitutional hepatic dysfunction. *Proc. Staff Meet. Mayo Clin.*, 10: 57, 1935.
2. Rozendaal, H. M., Comfort, M. W. and Snell, A. M. Slight and latent jaundice. *J. A. M. A.*, 104: 374, 1935.
3. Dameshek, W. and Singer, K. Familial nonhemolytic jaundice. *Arch. Int. Med.*, 67: 259–285, 1941.
4. Soffer, L. J. and Paulson, M. Comparative advantages and further modification of the bilirubin excretion test for hepatic function. *Am. J. M. Sc.*, 192: 535, 1936.
5. Weech, A. A., Vann, D. and Grillo, R. A. The clearance of bilirubin from the plasma. A measure of the excreting power of the liver. *J. Clin. Investigation*, 20: 326, 1941.
6. Comfort, M. W. and Hoyne, R. M. Constitutional hepatic dysfunction: clinical study of thirty-five cases. *Gastroenterology*, 3: 155, 1944.
7. Comfort, M. W. Constitutional hepatic dysfunction. *M. Clin. North America*, 29: 982, 1945.
8. Crigler, J. F. and Najjer, V. A. Congenital familial non-hemolytic jaundice with kernicterus. *Pediatrics*, 10: 169, 1952.
9. Curry, J. J. et al. Familial non-hemolytic jaundice. *New England J. Med.*, 226: 909, 1942.
10. Carithers, H. A. Non-hemolytic familial jaundice. *J. Pediat.*, 19: 817–821, 1941.

SMOOTHAGE ACTION IN CONSTIPATION

Roentgenographic pattern of colon mass propulsion:[1]

(1) Ascending colon filled.
(2) Unsegmented mass propelled through transverse colon.
(3) Propulsive force follows mass through descending colon.
(4) Pelvic colon reservoir filled.



Reestablishing Bowel Reflexes with Metamucil®

Nervous fatigue, tension, injudicious diet, failure to establish regularity, too little exercise, excessive use of cathartics—all factors which contribute to constipation.[2]

Sufficient bulk and sufficient fluid form the basic rationale of treatment of constipation with Metamucil.

Metamucil (the mucilloid of Plantago ovata) produces a bland, smooth bulk when mixed with the intestinal contents. This bulk, through its mass alone, stimulates the peristaltic reflex and thus initiates the desire to evacuate, even in patients in whom postoperative hesitancy exists.

Such gentle stimulation is of distinct advantage in reeducating and reestablishing those reflexes which control bowel evacuation. Many factors may pervert the normal reflexes, causing finally chronic constipation. Among them are: nervous fatigue and tension, improper intake of fluid, improper dietary habits, failure to respond to the call to stool, lack of physical exercise and abuse of the intestinal tract through excessive use of laxatives.[3]

Correction of constipation logically, therefore, lies in the suitable adjustment of these factors. The characteristics of Metamucil permit the correction of most of these factors: it provides bulk; it demands adequate intake of fluids (one glass with Metamucil powder, one glass after each dose); it increases the physiologic demand to evacuate; and it does not establish a laxative "habit." Metamucil, in addition, is inert, and also nonirritating and nonallergenic.

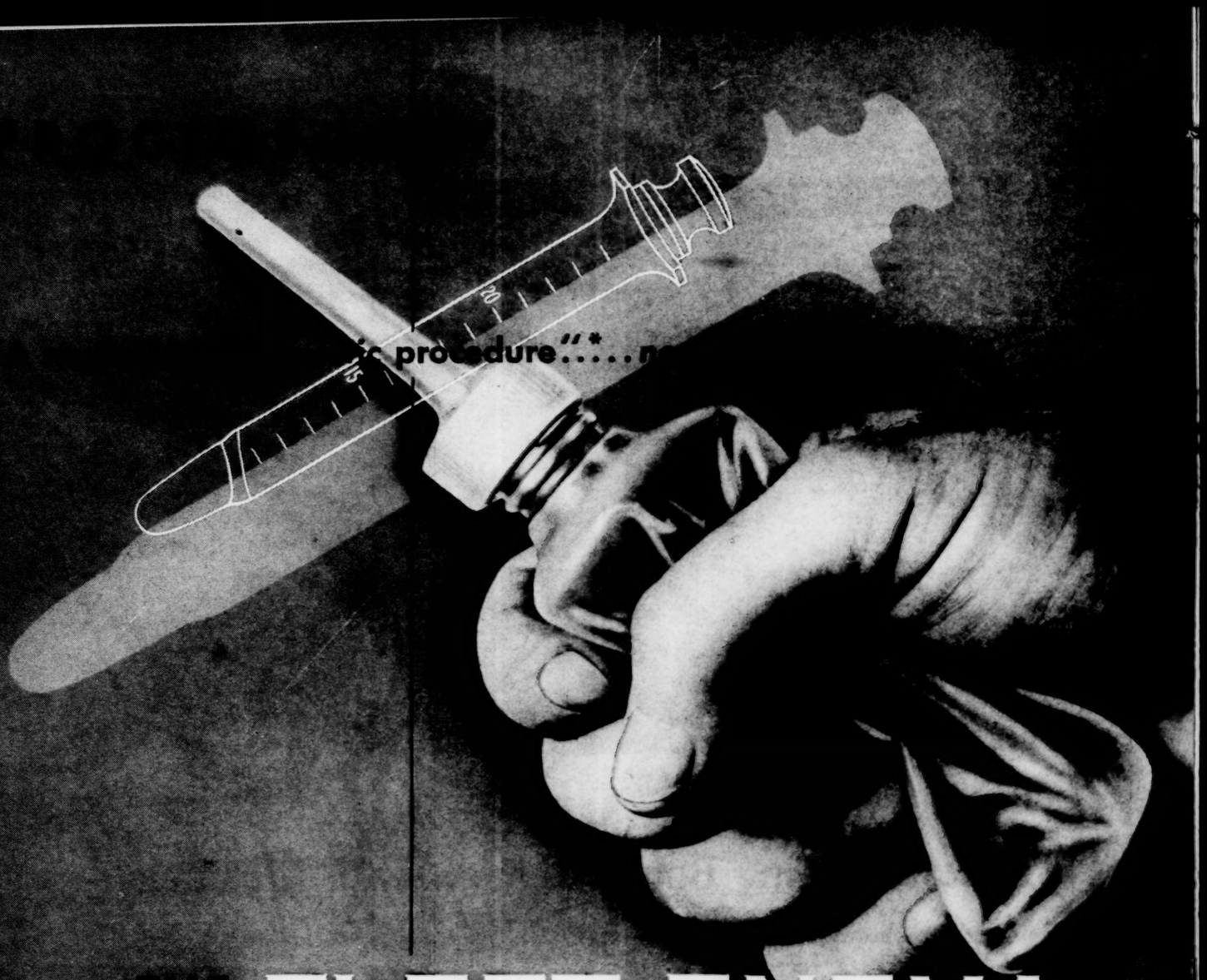
The average adult dose is one rounded teaspoonful of Metamucil powder in a glass of cool water, milk or fruit juice, followed by an additional glass of fluid if indicated.

Metamucil is the highly refined mucilloid of Plantago ovata (50%), a seed of the psyllium group, combined with dextrose (50%) as a dispersing agent. It is supplied in containers of 4, 8 and 16 ounces. G. D. Searle & Co., Research in the Service of Medicine.

---

1. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice: A Text in Applied Physiology, ed. 5, Baltimore, The Williams & Wilkins Company, 1950, pp. 579-583.
2. Bargen, J. A.: A Method of Improving Function of the Bowel, Gastroenterology *13*:275 (Oct.) 1949.

SEARLE



THE FLEET ENEMA

DISPOSABLE UNIT

"Probably no other office procedure ... in the adult gives as high a percentage of ...

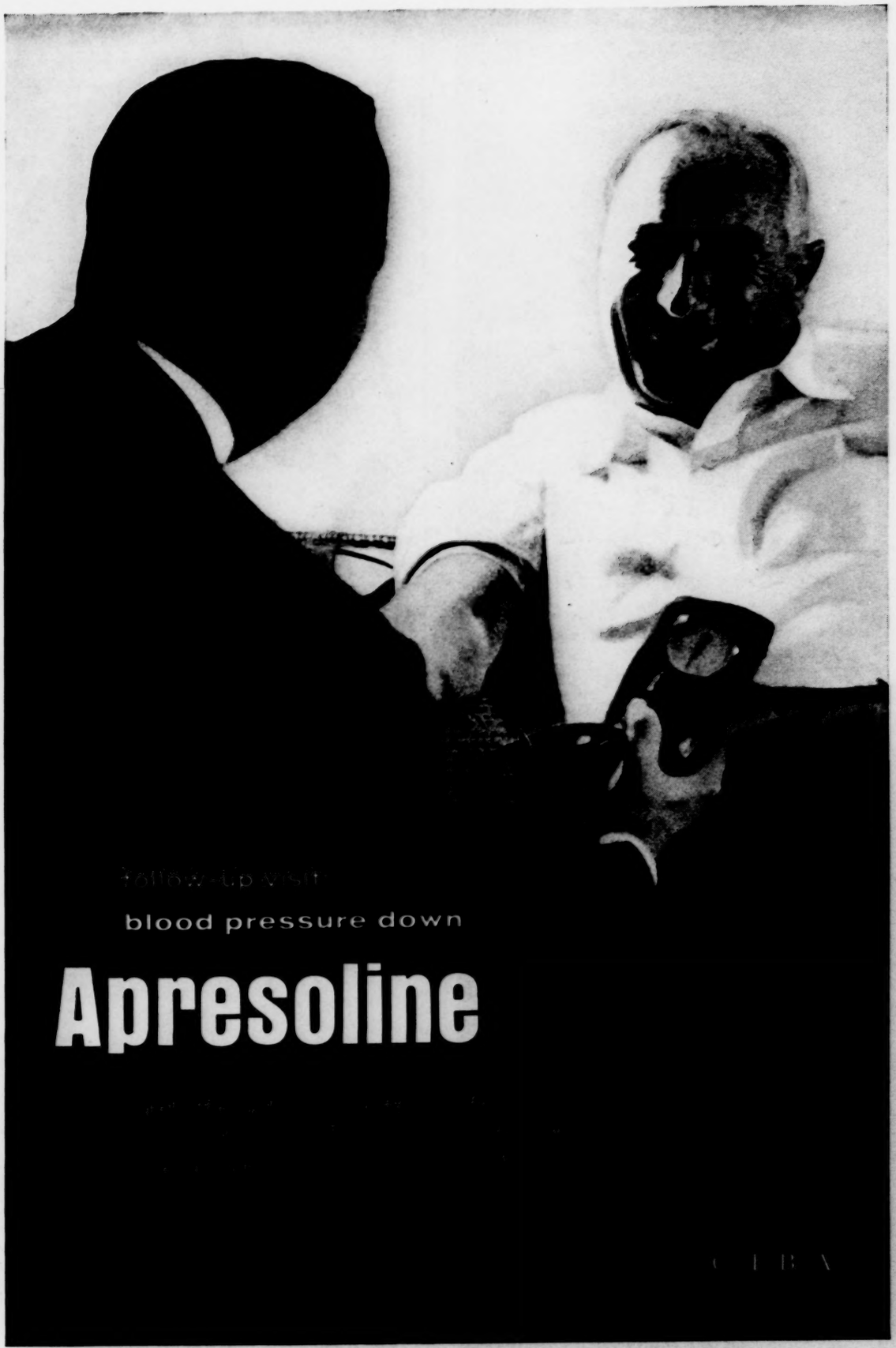
It is now a simple matter to prepare patients ... examination during an office visit. The Fleet ... Unit is superior in cleansing effect to a tap water or saline ... irritating than a soap suds enema. Thorough ... discomfort to the patient, is usually a matter of only four or five minutes.

Each 4½ fl. oz. disposable "squeeze bottle" contains, per 100 cc., 16 gm. sodium biphosphate and 6 gm. sodium phosphate . . . an enema solution of Phospho-Soda (Fleet) . . . gentle, prompt, thorough.

"Phospho-Soda", "Fleet" and "Fleet Enema" are registered trade-marks of C. B. Fleet Co., Inc.

Founded in 1869

C. B. FLEET CO., INC. • LYNCHBURG, VA.



MEDICAL HORIZONS TV Monday P.M. *Sponsored by* CIBA ABC-TV

your best bet!

NOW...
Federal law permits oral prescription

Hycodan®

(Dihydrocodeinone with Homatropine Methylbromide)

BETTER THAN CODEINE **FOR COUGH**[1]

Syrup and oral tablets. Each teaspoonful or tablet of HYCODAN contains 5 mg. dihydrocodeinone bitartrate and 1.5 mg. Mesopin. May be habit-forming. Average adult dose, 1 teaspoonful or 1 tablet after meals and at bedtime.

FASTER • LONGER-LASTING • MORE THOROUGH

BETTER THAN CODEINE *PLUS* APC **FOR PAIN**[2]

Percodan®

Salts of Dihydrohydroxycodeinone and Homatropine plus APC

Scored, yellow oral tablets. May be habit-forming. Average adult dose, 1 tablet q. 6 h.

Endo®

Literature Available

ENDO PRODUCTS INC.
RICHMOND HILL 18, NEW YORK

now available... 3 forms of

MYCOSTATIN

SQUIBB NYSTATIN

the first safe antifungal antibiotic

highly effective in vaginal moniliasis

Each vaginal tablet contains 100,000 units of Mycostatin and 0.95 Gm. of lactose. Packages of 15.

highly effective in monilial infections of the skin

100,000 units of Mycostatin per gram. 30 Gm. tubes.

highly effective in intestinal moniliasis; sometimes effective in generalized (systemic) moniliasis

Each tablet contains 500,000 units of Mycostatin. Bottles of 12 and 100.

Also available: broad spectrum antibacterial therapy plus prophylaxis against monilial superinfection

MYSTECLIN CAPSULES
250 mg. Steclin (Squibb Tetracycline) Hydrochloride and 250,000 units Mycostatin. Bottles of 12 and 100.

SQUIBB

602118

filmtab

Nembu-Serpïm

TRADEMARK

round-the-clock sedation plus tranquilization

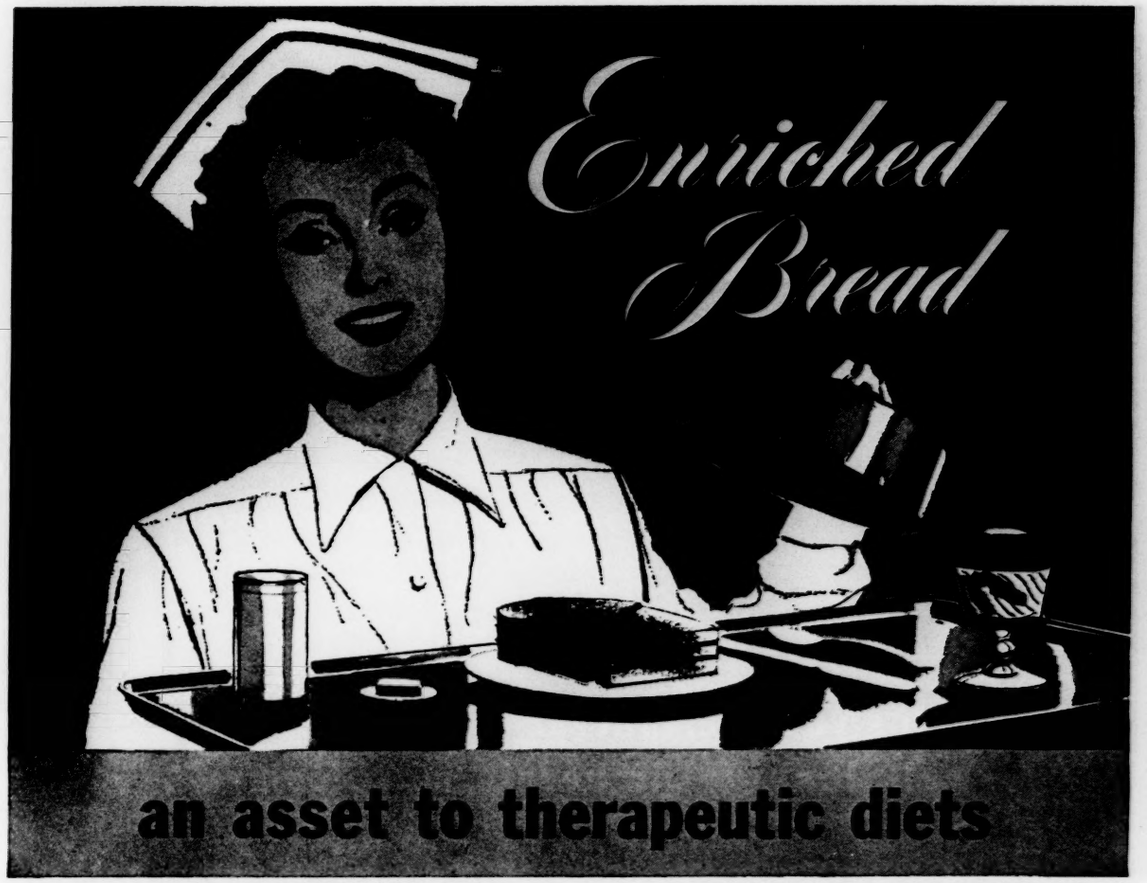
The combination of 30 mg. (½ gr.) Nembutal Calcium (Pentobarbital Calcium) and 0.25 mg. reserpine in each tiny Nembu-Serpin Filmtab provides longer-lasting sedation—without drowsiness— than Nembutal alone, plus a more rapid onset of tranquilizing effect than reserpine alone.

- short-acting Nembutal quickly induces drowsiness at bedtime, followed by refreshing sleep.
- longer-acting reserpine calms patients through the following days, yet they keep their drive and energy.

Faster-acting—no waiting for cumulative response. Anxiety and hypertension patients experience almost immediate relief as Nembu-Serpin's sedative-tranquilizing action rapidly takes effect. *Smaller dosages—fewer side effects.* Small dosages make side effects rare, medication economical, dosage schedules simple. Just one Nembu-Serpin Filmtab at bedtime will calm the worries of most anxiety patients. In bottles of 100 and 500 Filmtabs.

Abbott

©Film-sealed tablets.



Attention to the nutritional requirements of patients effectively supplements medical procedures in helping reduce mortality rates and in shortening convalescence. A state of good nutrition enhances resistance to disease, increases the capacity of tissue for repair, and promotes morale.

Nutritional Advantages

Because of its enrichment and its nonfat milk solids content, the average enriched bread supplies valuable amounts of good quality protein, thiamine, riboflavin, niacin, iron, and calcium. Its protein functions for growth, repair, and maintenance. Its calories help to spare protein for specific protein uses and contribute to energy needs.

The table (right) points up how effectively 6 slices participate in providing good nutrition in illness and convalescence.

Physiologic Advantages

Soft and open in texture, enriched bread is easily masticated and swallowed. It is promptly and thoroughly digested. Its appetizing eating qualities reflexly incite the digestive processes. Producing insignificant amounts of smooth inert residue, it does not irritate the gastric or intestinal mucosa.

Dietetic Advantages

In either fresh or toasted form, enriched bread adds to the eating pleasure of meals. Neutral in flavor, it blends well with other foods. When appetite lags, sandwiches including a wide variety of foods—meat, poultry, eggs, cheese, salad preparations and various spreads—give zest to eating as well as needed nourishment.

These advantages—nutritional, physiologic, and dietetic—establish enriched bread as a valuable asset in therapeutic diets.

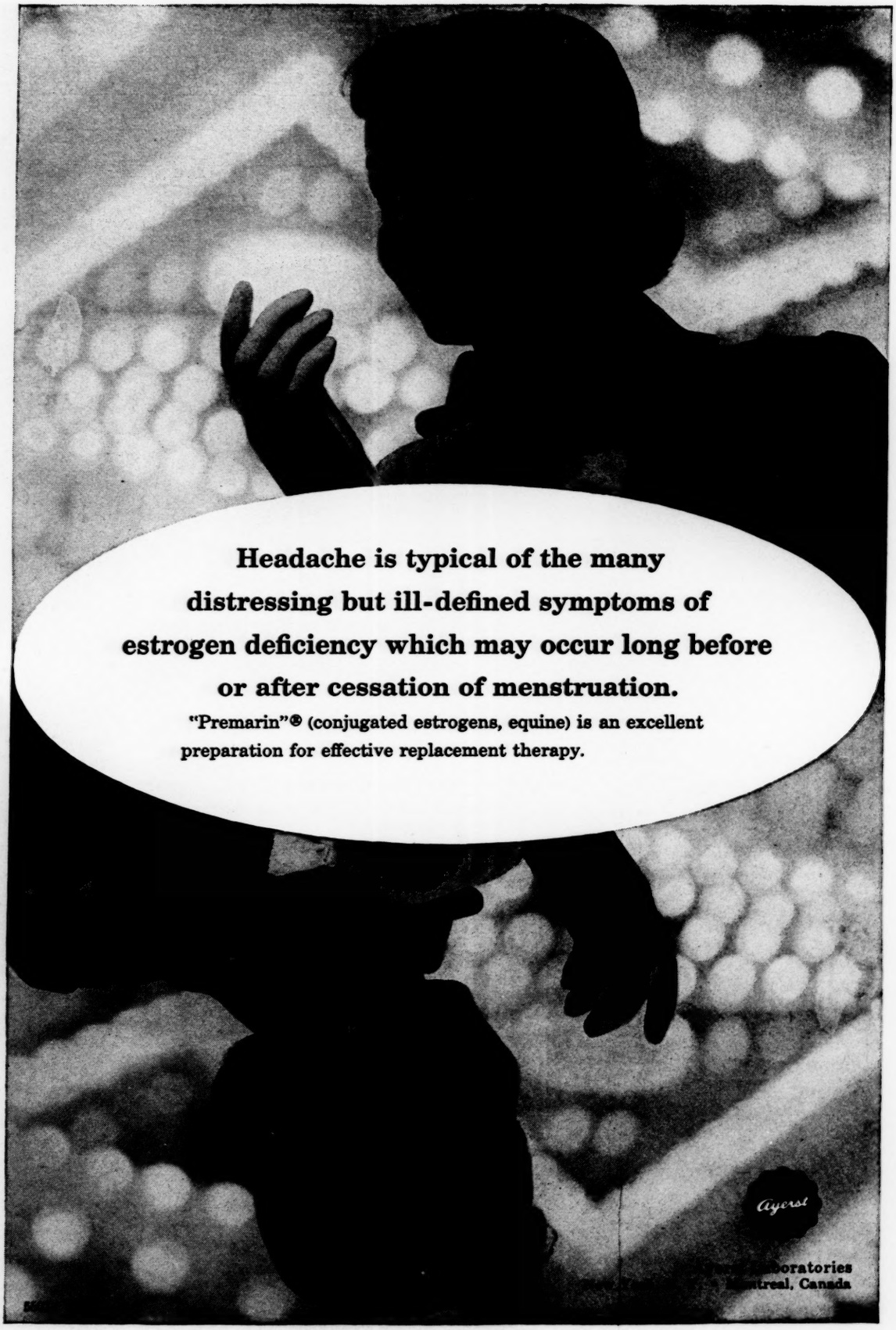
Contribution of 6 Slices of Enriched Bread

	Nutrients and Calories	Percentages of Allowances*
Protein	11.7 Gm.	18%
Thiamine	0.33 mg.	22
Niacin	3.0 mg.	20
Riboflavin	0.21 mg.	13
Iron	3.3 mg.	28
Calcium (average)	122 mg.	15
Calories	379	13

*Percentages of daily allowances for 143 lb., 67 in. tall fairly active man of 45. Recommended Dietary Allowances, Washington, D.C., National Academy of Sciences—National Research Council, Publication 302, 1953.

The nutritional statements made in this advertisement have been reviewed and found consistent with current medical opinion by the Council on Foods and Nutrition of the American Medical Association.

AMERICAN BAKERS ASSOCIATION 20 North Wacker Drive • Chicago 6, Illinois


Headache is typical of the many distressing but ill-defined symptoms of estrogen deficiency which may occur long before or after cessation of menstruation.
"Premarin"® (conjugated estrogens, equine) is an excellent preparation for effective replacement therapy.
Ayerst
oratories
treal, Canada

Doctor, do you feel that your colleagues are missing important new medical findings by not reading The American Journal of Surgery?

Let us send them a complimentary copy—of course there is no obligation to you or your colleagues. Just fill out the coupon and mail it to us.

*Dr.*_____ *Dr.*_____

*Address*_____ *Address*_____

_____ _____

*Dr.*_____ *Your Name*_____

*Address*_____ *Address*_____

_____ _____

A.J.M.-2

The American Journal of Medicine

49 West 45th Street, · New York 36, New York

HEDULIN®

(Phenindione Walker)

". . . . . *it is our feeling that, at present, this [phenindione] is the oral anticoagulant of choice.*"

Breneman, G. M., and Priest, E. McC.: Am. Heart J. 50:129-35, 1955.

". . . . . *phenindione seems to be a more satisfactory anticoagulant at this time.*"

Wood, J. E., Jr.; Beckwith, J. R., and Camp, J. L.: J.A.M.A. 159:635, 1955.

HEDULIN permits dependable prothrombin control with little risk of dangerous fluctuation

(Not a Coumarin Drug)

DOSAGE 4 to 10 tablets (200 to 500 mg.) initially, half in the morning, and half at night; maintenance dosage (on basis of prothrombin determination daily for first 3 days), 50 to 100 mg. daily, divided as above.

AVAILABLE on prescription through all pharmacies in original bottles of 100 and 1,000 scored tablets (50 mg. each).

HEDULIN is not cumulative in effect — provides greater uniformity of action and ease of maintenance.

HEDULIN is rapidly excreted — therapeutic effect dissipated within 24-48 hours, if withdrawal becomes necessary.

HEDULIN acts promptly — producing therapeutic prothrombin levels in 18-24 hours.

HEDULIN requires fewer prothrombin determinations—only one every 7-14 days after maintenance dose is established.

HEDULIN's anticoagulant action is rapidly reversed by vitamin K_1 emulsion.

WRITE FOR LITERATURE AND TRIAL SUPPLIES

Walker LABORATORIES, INC.

MOUNT VERNON, NEW YORK, U.S.A.

S & H—7043—SS1201

Pork in the Human Dietary

PORK MAY be looked upon as an important factor in America's general health and well-being. The average intake of pork in America is about 46 pounds of lean pork and 20 pounds of bacon and salt pork per person each year.[1] But America's demand for pork goes further than taste appeal and deeper than mere statistics. Pork makes a valuable contribution to day-in-and-day-out nutrition.

Pork rates among the foremost sources of thiamine. As a source of all other B vitamins and many essential minerals, such as iron and phosphorus, pork meat is considered an important dietary constituent.

Lean pork is virtually completely digestible. Its protein serves to promote growth and aid in the maintenance of tissue cells. Like all high quality protein, that of pork aids in the elaboration of protein hormones, enzymes, and antibodies.

Pork constitutes a valuable part of the daily diet (Table I), and also contributes importantly to the nutrition of the pregnant woman (Table II).

Pork and pork products have won America's favor by their unique combination of econcmy, palatability, and nutritional value.

---

1. Consumption of Food in the United States, 1909-1952, Washington, D.C., United States Department of Agriculture, Bureau of Agricultural Economics, Agricultural Handbook No. 62, September, 1953.

2. Watt, B.K., and Merrill, A.L.: Composition of Foods—Raw, Processed, Prepared, Washington, D.C., United States Department of Agriculture, Agr cultural Handbook No. 8, 1950.

3. Bowes, A. deP., and Church, C.F.: Food Values of Portions Commonly Used, ed. 7, Philadelphia, Anna dePlanter Bowes, 1951.

4. Cheldelin, V.H., and Williams, R.J.: Studies on the Vitamin Content of Tissues, II, Houston, Texas, University of Texas Publication No. 4237, 1942.

5. Schweigert, B.S.; Nielsen, E.; McIntire, J.N., and Elvehjem, C.A.: Biotin Content of Meat and Meat Products, J. Nutrition *26*:65 (July) 1943.

6. Scheid, H.E., and Schweigert, B.S.: The Vitamin B_{12} Content of Meat, Annual Report, An Outline of Research During the Fiscal Year 1953-54, Chicago, American Meat Institute Foundation, Bull. 22, 1955.

7. Estimated on basis of protein content of meats. Sherman H.C.: Food Products, ed. 4, New York, The Macmillan Company, 1948 p. 155.

8. Recommended Dietary Allowances, Washington, D.C., National Academy of Sciences—National Research Council, Publication 302, 1953.

TABLE I Cooked Pork Chops, Ham, and Pork Sausage
Nutrients and Calories Provided by 3-Ounce Portions

	Protein Gm.	Thiamine mg.	Niacin mg.	Riboflavin mg.	Iron mg.	Phosphorus mg.	Calories
Pork Chops, without bone, cooked, 3 oz.[2]	20	0.71	4.3	0.20	2.6	200	284
Ham, without bone, cooked, 3 oz.[2]	20	0.45	4.0	0.20	2.6	202	338
Pork Sausage, cooked, 3 oz.[3]	14	0.42	2.8	0.20	2.1	139	396

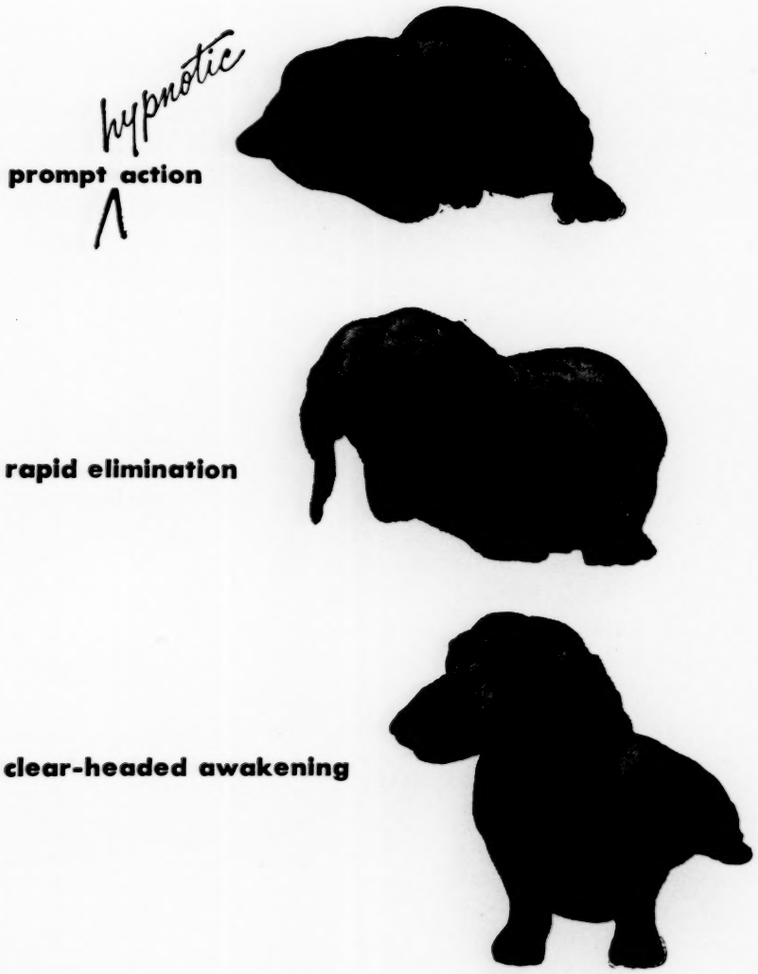
3.5 ounces of fresh pork loin, equivalent to approximately 3 ounces of cooked loin, contains 0.47 mg. pantothenic acid;[4] 0.10 mg. pyridoxine;[4] 0.005 mg. biotin;[5] 36 mg. inositol;[4] 0.08 mg. folic acid;[4] 0.0027 mg. vitamin B_{12};[6] 63 mg. chlorine;[7] 0.1 mg. copper;[7] 20 mg magnesium;[7] 280 mg. potassium;[7] 70 mg. sodium;[7] and 0.01 mg. manganese.[7]

TABLE II Nutrients and Calories of Cooked Pork Chops (3 ounces) Expressed as Percentages of Recommended Daily Dietary Allowances[8]

Percentages of Allowances for:	Protein	Thiamine	Niacin	Riboflavin	Iron	Phosphorus	Calories
Girls, 13-15 years of age; weight, 108 lb.; height, 63 inches.	25%	55%	33%	10%	17%	15%	11%
Women, 25 years of age; weight, 121 lb.; height, 62 inches.	31%	59%	36%	14%	22%	17%	12%
Pregnant Women (3rd trimester)	25%	47%	29%	10%	17%	13%	11%

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

American Meat Institute
Main Office, Chicago... Members Throughout the United States

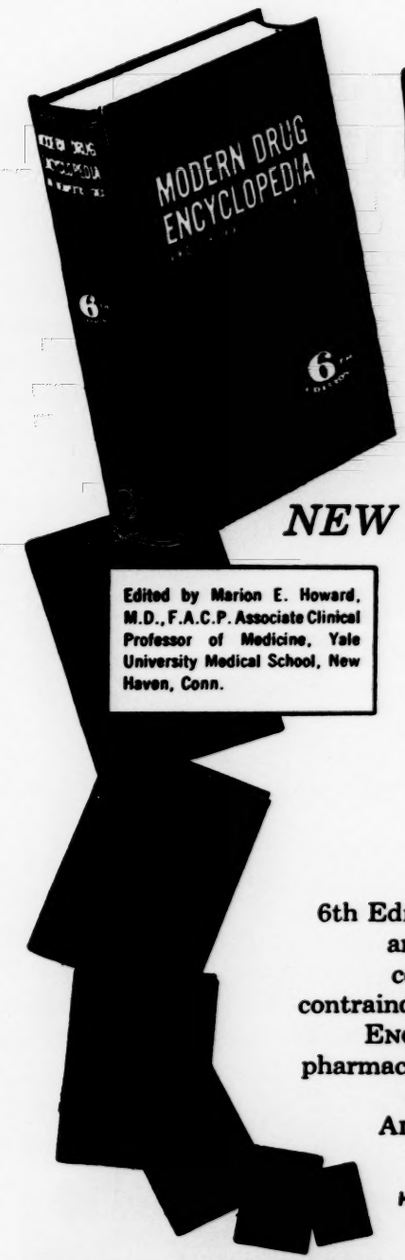


ELIXIR ALURATE

'Roche'

Available as ELIXIR ALURATE, cherry red color/ELIXIR ALURATE VERDUM, emerald green color
Each contains 0.03 Gm (½ grain) of Alurate per teaspoonful (4 cc)
in a palatable vehicle. Alurate®—brand of aprobarbital

HOFFMANN-LA ROCHE INC. • ROCHE PARK • NUTLEY 10 • NEW JERSEY



Better than Ever...

for complete descriptions and authoritative data on nearly 4000 modern ethical drugs

NEW 6th *edition* **MODERN DRUG ENCYCLOPEDIA** *and* **THERAPEUTIC INDEX**

Edited by Marion E. Howard, M.D., F.A.C.P. Associate Clinical Professor of Medicine, Yale University Medical School, New Haven, Conn.

with FREE Bi-monthly Supplement MODERN DRUGS to keep you up-to-date with newest ethical drug descriptions

The only three-year reference service of its kind with complete, authoritative data on new ethical drugs now completely revised in this new, "better than ever", 6th Edition. Prescription products, narcotics and exempt-narcotics are indicated for the first time. Here is your source for latest composition, action, uses, supply, dosage—also cautions and contraindications of thousands of new drugs. The MODERN DRUG ENCYCLOPEDIA is the leading finger-tip reference for physician, pharmacist, drug room and hospital infirmary, college and university. Compiled in seven special sections: DRUGS • BIOLOGICALS ALLERGENS • GENERIC NAME INDEX • THERAPEUTIC INDEX MANUFACTURER'S INDEX • GENERAL INDEX

HANDSOMELY BOUND IN RED FABRICOID
CONTAINS 1486 PAGES, SIZE 6" x 9¼" x 2¼"

COMPLETE WITH GENERIC NAME INDEX AND SELF-PRONOUNCING DRUG LISTINGS

- ★ Over 50,000 Physician, Pharmacist, Institution users!
- ★ Now required reference by Michigan State Board of Pharmacy
- ★ Recognized as leading reference text by College and University Schools of Pharmacy
- ★ 97.2% subscribers who receive it . . . use it
- ★ 89.8% keep it within finger-tip reach

MAIL THIS COUPON NOW

DRUG PUBLICATIONS, INC.
49 West 45th Street, New York 36, N. Y.

Enclosed is the sum of fifteen dollars ($15.00** U.S.A.) for which please send me postpaid the Sixth Edition of the MODERN DRUG ENCYCLOPEDIA AND THERAPEUTIC INDEX plus the bi-monthly supplementary service, MODERN DRUGS.

NAME_____

ADDRESS_____

CITY_____ ZONE____ STATE_____

**Foreign $18.00 **Includes three-year supplementary service at $3 per year.

M26

stable

RAUDIXIN

SQUIBB WHOLE ROOT RAUWOLFIA

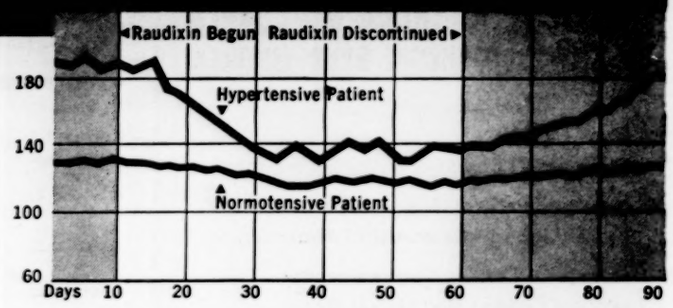
in anxiety and tension states . . .

stable tension

in hypertension . . .

stable hypertension

Comparative effect of Raudixin on the blood pressure of hypertensive patient and normotensive patient.



The hypotensive action of Raudixin is selective for the hypertensive state. For this reason, Raudixin does not significantly affect the blood pressure of normotensive patients.

DOSAGE: 100 mg. b.i.d. initially; may be adjusted within a range of 50 mg. to 500 mg. daily. Most patients can be adequately maintained on 100 mg. to 200 mg. daily.

SUPPLY: 50 mg. and 100 mg. tablets, bottles of 100, 1000 and 5000.

SQUIBB

Advertisers Index

February, 1956